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FILE 'CAPLUS' ENTERED AT 09:16:41 ON 17 SEP 2002

=> S STEREOSELECTIVITY;S REGIOSELECTIVITY;S STEREO?;S REGIO?;S POLYMERASE CHAIN REACTION;S PCR
         15248 STEREOSELECTIVITY
          1080 STEREOSELECTIVITIES
L1        15909 STEREOSELECTIVITY
                (STEREOSELECTIVITY OR STEREOSELECTIVITIES)


          9629 REGIOSELECTIVITY
           546 REGIOSELECTIVITIES
L2         9917 REGIOSELECTIVITY
                (REGIOSELECTIVITY OR REGIOSELECTIVITIES)


L3       197779 STEREO?


L4      1091180 REGIO?


         124183 POLYMERASE
           7824 POLYMERASES
         125347 POLYMERASE
                (POLYMERASE OR POLYMERASES)
         521126 CHAIN
         258614 CHAINS
         673489 CHAIN
                (CHAIN OR CHAINS)
        2480216 REACTION
        1782177 REACTIONS
        3321578 REACTION
                (REACTION OR REACTIONS)
L5       75940 POLYMERASE CHAIN REACTION
                (POLYMERASE(W)CHAIN(W)REACTION)


         107967 PCR
            588 PCRS
L6       108064 PCR
                (PCR OR PCRS)

=> S (L3,L4) AND (L5,L6)
L7       29926 ((L3 OR L4)) AND ((L5 OR L6))

=> S MAGNESIUM OR MG;S MANGANESE OR MN;S DEOXYNUCLEOTIDES;S DATP;S DCTP;S DGTP;S DTTP
         328381 MAGNESIUM
             85 MAGNESIUMS
         328423 MAGNESIUM
                (MAGNESIUM OR MAGNESIUMS)
        1217210 MG
           1068 MGS
        1217899 MG
                (MG OR MGS)
L8      1399009 MAGNESIUM OR MG


         265029 MANGANESE
            102 MANGANESES
         265040 MANGANESE
                (MANGANESE OR MANGANESES)
         365860 MN
           4230 MNS
         368287 MN
                (MN OR MNS)
L9       489957 MANGANESE OR MN


L10         795 DEOXYNUCLEOTIDES
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          2702 DATP
             2 DATPS
L11       2702 DATP
                (DATP OR DATPS)


          1979 DCTP
             8 DCTPS
L12       1985 DCTP
                (DCTP OR DCTPS)


          1675 DGTP
             1 DGTPS
L13       1676 DGTP
                (DGTP OR DGTPS)


          1914 DTTP
             9 DTTPS
L14       1915 DTTP
                (DTTP OR DTTPS)

=> S L5,L6
L15     130419 (L5 OR L6)

=> S L15 AND L8;S L15 AND L9;S L15 AND L10;S L15 AND (L11,L12,L13,L14)
L16       3791 L15 AND L8


L17        451 L15 AND L9


L18         38 L15 AND L10


L19        418 L15 AND ((L11 OR L12 OR L13 OR L14))

=> S MUTATION
        172273 MUTATION
        110853 MUTATIONS
L20     214017 MUTATION
                (MUTATION OR MUTATIONS)

=> S L7 AND L20
L21       6412 L7 AND L20

=> S (L1,L2) AND (L5,L6)
L22         20 ((L1 OR L2)) AND ((L5 OR L6))

=> D 1-20 CBIB ABS

L22  ANSWER 1 OF 20  CAPLUS  COPYRIGHT 2002 ACS
2002:668343  A desaturase-like protein from white spruce is a .DELTA.9
     desaturase.  Marillia, Elizabeth-France; Giblin, E. Michael; Covello,
     Patrick S.; Taylor, David C. (Seed Oil Biotechnology Group, National
     Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium
     Place, Saskatoon, SK, S7N 0W9, Can.).  FEBS Letters, 526(1-3), 49-52
     (English) 2002.  CODEN: FEBLAL.  ISSN: 0014-5793.  Publisher: Elsevier
     Science B.V..
AB   Gymnospermae seed lipids are characterized by a high degree of desatn.,
     most having a .DELTA.9 double bond.  By degenerate   ***polymerase***
       ***chain***     ***reaction***   (  ***PCR***  ) we have isolated a white
     spruce (Picea glauca) cDNA clone that encodes an amino acid sequence
     sharing a high degree of homol. with other putative plant acyl-CoA (CoA)
     Des9 desaturases.  Both in vivo and in vitro expression studies in a
     .DELTA.9 desaturase-deficient yeast strain demonstrated the desatn.
     functionality of the white spruce clone, and gas chromatog.-mass
     spectrometry (GC-MS) analyses confirmed the   ***regioselectivity***   of
     the encoded enzyme.  This is the first report of the functional
     characterization of a plant membrane-bound acyl-CoA-like protein .DELTA.9
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desaturase by heterologous expression in yeast.

L22 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS
2001:296290 Document No. 134:361661 Expression of GluR6/7 subunits of kainate receptors in rat adenohypophysis. Hinoi, Eiichi; Yoneda, Yukio (Department of Molecular Pharmacology, Faculty of Pharmaceutical Sciences, Kanazawa University, Ishikawa, 920-0934, Japan). Neurochemistry International, 38(6), 539-547 (English) 2001. CODEN: NEUIDS. ISSN: 0197-0186. Publisher: Elsevier Science Ltd..
AB We have previously demonstrated the presence of unidentified [3H]glutamate (Glu) binding sites with ***stereoselectivity*** , high affinity and saturability in rat peripheral excitable tissues such as the pituitary. In this study, peripheral binding sites were further evaluated for the ionotropic Glu receptor subtype insensitive to N-methyl-D-aspartate by using reverse transcription ***polymerase*** ***chain*** ***reaction*** (RT- ***PCR***) and Western blotting, in addn. to receptor binding using radiolabeled ligands other than [3H]Glu. Binding of [3H]kainate (KA) and [3H]DL-.alpha.-amino-3-hydroxy-5-methylisoxazole-4-propionate was detected in membrane prepns. obtained from the rat pituitary and adrenal irresp. of prior treatment with Triton X-100. An RT- ***PCR*** anal. revealed constitutive expression of mRNA for GluR1, GluR3, GluR5, KA1 and KA2 subunits in the rat adrenal and pituitary, as well as the brain and retina. The pituitary also expressed mRNA for GluR2, GluR4, GluR6 and GluR7 subunits in contrast to the adrenal. Under our exptl. conditions employed, however, Western blotting assays failed to confirm the expression of receptor proteins for GluR1, GluR2/3 and GluR4 subunits in the adrenal cortex, adrenal medulla, adenohypophysis and neurohypophysis. Immunoreactive GluR6/7 subunits were only detectable in the adenohypophysis, but not in the adrenal cortex, adrenal medulla and neurohypophysis. An i.p. injection of KA doubled DNA binding activity of the nuclear transcription factor activator protein-1 in the rat pituitary, with concomitant more potent potentiation of that in the hippocampus. These results suggest that GluR6/7 subunits of KA receptors may be constitutively expressed with responsiveness to the systemic administration of an agonist at least in the rat adenohypophysis.

L22 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2002 ACS
2000:686338 Document No. 133:263209 Stereoselective transaminase, gene encoding said protein and use for producing optically active amino compounds. Takashima, Yoshiki; Mitsuda, Satoshi; Wieser, Marco (Sumitomo Chemical Company, Limited, Japan). Eur. Pat. Appl. EP 1038953 A1 20000927, 54 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2000-400701 20000314. PRIORITY: JP 1999-75511 19990319; JP 1999-88634 19990330.
AB According to the present invention, a novel protein capable of catalyzing transamination stereoselectively and a gene encoding said protein can be provided. Thus, the gene encoding a 37-kDa transaminase enzyme was cloned from Mycobacterium aurum SC-S423 and shown to encode a 339-amino acid product. The purified enzyme catalyzes transamination between acetophenone and racemic sec-butylamine to product 100% optically active (R)-1-phenylethylamine; decreased ***stereoselectivity*** is also demonstrated with 1-(3,4-dichlorophenyl)ethylamine and 1-(3,4-dimethoxyphenyl)-2-aminopropane. Thus, the Mycobacterium transaminase can be used for the synthesis of optically active amino compds.

L22 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS
2000:446380 Document No. 133:204554 A bifunctional .DELTA.6-fatty acyl acetylenase/desaturase from the moss Ceratodon purpureus a new member of the cytochrome b5 superfamily. Sperling, Petra; Lee, Michael; Girke, Thomas; Zahringer, Ulrich; Stymne, Sten; Heinz, Ernst (Institut fur Allgemeine Botanik, Universitat Hamburg, Germany). European Journal of Biochemistry, 267(12), 3801-3811 (English) 2000. CODEN: EJBCAI. ISSN: 0014-2956. Publisher: Blackwell Science Ltd..
AB Many plant genes have been cloned that encode regioselective desaturases catalyzing the formation of cis-unsatd. fatty acids. However, very few genes have been cloned that encode enzymes catalyzing the formation of the functional groups found in unusual fatty acids (e.g. hydroxy, epoxy or acetylenic fatty acids). Here, we describe the characterization of an acetylenase from the moss Ceratodon purpureus with a

regioselectivity differing from the previously described .DELTA.12-acetylenase. The gene encoding this protein, together with a .DELTA.6-desaturase, was cloned by a ***PCR*** -based approach with primers derived from conserved regions in .DELTA.5-, .DELTA.6-fatty-acid desaturases and .DELTA.8-sphingolipid desaturases. The proteins that are encoded by the two cloned cDNAs are likely to consist of a N-terminal extension of unknown function, a cytochrome b5-domain, and a C-terminal domain that is similar to acyl lipid desaturases with characteristic histidine boxes. The proteins were highly homologous in sequence to the .DELTA.6-desaturase from the moss Physcomitrella patens. When these two cDNAs were expressed in Saccharomyces cerevisiae, both transgenic yeast cultures desatd. .DELTA.9-unsatd. C16- and C18-fatty acids by inserting an addnl. .DELTA.6-cis-double bond. One of these transgenic yeast clones was also able to introduce a .DELTA.6-triple bond into .gamma.-linolenic and stearidonic acid. This resulted in the formation of 9,12,15-(Z,Z,Z)-octadecatrien-6-ynoic acid, the main fatty acid found in C. purpureus. These results demonstrate that the .DELTA.6-acetylenase from C. purpureus is a bifunctional enzyme, which can introduce a .DELTA.6-cis-double bond into 9,12,(15)-C18-polyenoic acids as well as converting a .DELTA.6-cis-double bond to a .DELTA.6-triple bond.

L22 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2002 ACS

2000:327261 Periplasmic expression of subtilisin E in E. coli for generation of mutants with novel characteristics (directed evolution).. Sroga, Grazyna E.; Dordick, Jonathan S. (Department of Chemical Engineering, Rensselaer Polytechnic Institute, Troy, NY, 12180-3590, USA). Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000, BIOT-041. American Chemical Society: Washington, D. C. (English) 2000. CODEN: 69CLAC.

AB We are interested in selection of subtilisin mutants that will perform synthesis of sugar esters (or sugar ester polymers) with controlled ***regioselectivity*** . This is an extremely difficult task for many reasons; the most crit. of which is a selection system that will allow us identification of mutants with different catalytic and regioselective properties. Since Bacillus subtilis uses sucrose, we switched to an E. coli host system that does not utilize sucrose as a carbon source. Another significant problem is that expression of active proteases, in our case serine protease, in E. coli could cause serious constrains for the organism survival. For this reason, we chose a tightly regulated prokaryotic expression system pBAD/gIII (Invitrogen), which allows periplasmic expression of recombinant proteins. Three subtilisin E genes, WT and two mutants (the DMF mutant and the temp. resistant mutant), were recloned into the pBAD/gIII expression vector. To generate secreted, recombinant proteins that are expressed correctly and contain the C-terminal fusion peptide, ***PCR*** was used. The cloning of the genes in the correct frame was confirmed by sequencing of both strands of each DNA construct. The resp. plasmids were transformed into E. coli TOP10 [F- mcrA .DELTA.(mrr-hsdRMS-mcrBC) .PHI.80lacZ.DELTA.M15 .DELTA.lacX74 deoR recA1 araD139 .DELTA.(araA-leu)7697 galU galK rpsL endA1 nupG]. Since each recombinant protein has different characteristics, expression and secretion are often problematic. Addnl. problems can emerge in the case of subtilisin E, because the protein is first synthesized as a precursor with a leader sequence. This sequence is essential for the formation of enzymically active subtilisin. We expressed functional subtilisin E in the E. coli periplasm. Addn. of the myc epitope and 6xHis tag at the C-terminus did not interfere with the activity of the protease. When viable E. coli cells expressing recombinant subtilisin E were suspended directly in the assay buffer, a strong color reaction occurred within 10min. To 15 min. Of addn. of the subtilisin E substrate (N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide). We use in vitro DNA Recombination technique as well as Mutagenic ***PCR*** for a generation of randomly mutated subtilisin E genes.

L22 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2002 ACS

2000:252424 Document No. 133:27755 A Novel Approach for Analyzing the Structure of DNA Modified by Benzo[a]pyrene Diol Epoxide at Single-Molecule Resolution. Pietrasanta, Lia I.; Smith, Bettye L.; MacLeod, Michael C. (Department of Physics, University of California, Santa Barbara, CA, 93106, USA). Chemical Research in Toxicology, 13(5), 351-355 (English) 2000. CODEN: CRTOEC. ISSN: 0893-228X. Publisher: American Chemical Society.

AB Benzo[a]pyrene diol epoxide (BPDE) has been shown to bind specifically to the exocyclic amino group of deoxyguanosine in duplex DNA. Interestingly, this metabolite exhibits ***stereoselectivity*** in its tumorigenic and mutagenic effects. It is thought that local DNA conformation is altered at the site of the adduct, resulting in aberrant biol. processes, and that in certain sequence contexts BPDE-DNA adducts induce bends in the DNA. In the work presented here, we compared DNA structural alterations of BPDE-modified DNA and unmodified DNA via tapping mode at. force microscopy (AFM). DNA fragments 366 base pairs (bp) in length were generated by ***PCR*** from the duplicated multiple-cloning site of pBEND2 inserted into pGEM-3Zf(-), and either mock-modified or treated with BPDE to give modification levels between 1 and 5% of the nucleotides. Control or BPDE-modified DNA was adsorbed to mica and visualized in air by AFM. The contour lengths and end-to-end lengths of individual mols. were measured. The ratio of end-to-end distance to contour length was significantly smaller for modified DNA mols. than for the unmodified DNA prepn., although the frequency distributions of the contour lengths were similar for the two prepns. This suggests BPDE-DNA adducts cause significant bending of DNA mols., confirming previous conclusions based on more indirect measurements. The av. induced bend angle for BPDE-DNA adducts is estd. to be at least 30.degree..

L22 ANSWER 7 OF 20 CAPLUS COPYRIGHT 2002 ACS

2000:44818 Document No. 132:205796 A monocarboxylate transporter MCT1 is located at the basolateral pole of rat jejunum. Orsenigo, M. N.; Tosco, M.; Bazzini, C.; Laforenza, U.; Faelli, A. (Dipartimento di Fisiologia e Biochimica Generali, Universita di Milano, Milan, I-20133, Italy). Experimental Physiology, 84(6), 1033-1042 (English) 1999. CODEN: EXPHEZ. ISSN: 0958-0670. Publisher: Cambridge University Press.

AB We have functionally expressed and identified a monocarboxylate transporter (MCT1) from rat jejunal enterocyte and we provide evidence for its basolateral localization. Poly(A)+ RNA isolated from rat jejunum was injected into Xenopus laevis oocytes and expression of a proton-lactate symporter was investigated by means of L-[14C]lactate uptake. The existence of an endogenous capacity for L-lactate transport was demonstrated; when, however, oocytes were injected with jejunal mRNA, an expressed L-lactate uptake was seen which differed from the endogenous transporter since it was significantly pH dependent. After sucrose d. gradient fractionation, the highest expression of the pH-dependent lactate uptake was detected with the mRNA size fraction of about 2-3 kb in length. The substrate specificity, ***stereoselectivity*** and sensitivity to pCMBS (an organomercurial thiol reagent that modifies cysteine residues) of the expressed transport were in good agreement with results previously obtained using isolated jejunal basolateral membranes. Using the reverse transcriptase- ***polymerase*** ***chain*** ***reaction*** , the presence of mRNA coding for the MCT1 isoform was demonstrated in jejunal enterocytes. These data, together with previous results, suggest that MCT1 is a major route for lactate efflux across the basolateral membrane of rat jejunum; this is in contrast to current opinion which restricts the presence of MCT1 to the apical membrane of the whole small intestine.

L22 ANSWER 8 OF 20 CAPLUS COPYRIGHT 2002 ACS

1999:111358 Document No. 130:333468 Structure and Expression of the Rat CYP3A1 Gene: Isolation of the Gene (P450/6.beta.B) and Characterization of the Recombinant Protein. Nagata, Kiyoshi; Ogino, Makoto; Shimada, Miki; Miyata, Masaaki; Gonzalez, Frank J.; Yamazoe, Yasushi (Division of Drug Metabolism and Molecular Toxicology, Tohoku University, Sendai, 980-8578, Japan). Archives of Biochemistry and Biophysics, 362(2), 242-253 (English) 1999. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB A P450 gene (P450/6.beta.B) of the CYP3A subfamily was isolated from a rat genomic library. Nucleotide sequencing of the exons revealed a high similarity with P450PCN1 cDNA (Gonzalez et al. 1985, J. Biol. Chem. 260, 7345-7441), but differed in 41 nucleotides, resulting in 11 changes and 2 deletions of amino acid residues. The P450/6.beta.B spanned about 30 kbp and consisted of 13 exons, and was in exon no. and size identical with CYP3A2 gene except in the 6th exon, which was shorter than that of CYP3A2. 6.beta.-B mRNA, which may be transcribed from P450/6.beta.B, was detected on Northern blotting and by reverse transcription- ***polymerase*** ***chain*** ***reaction*** (RT- ***PCR***). Profiles of the developmental change and induction by a treatment with several chems. were

very similar to those of P450PCN1 mRNA reported previously. P450PCN1 mRNA and gene, however, were not detected by ***PCR*** in rats. To det. whether P450/6.beta.B encodes an active protein, a cDNA was isolated and expressed. Expression of 6.beta.-B cDNA in COS-1 cells was carried out and revealed that the recombinant protein comigrated with purified P4506.beta.-4 previously identified as CYP3A1. The recombinant 6.beta.-B protein showed similar turnover rate and ***regioselectivity*** for testosterone with purified P4506.beta.-4 by the simultaneous addn. of NADPH-cytochrome P 450 reductase and cytochrome b5. These data suggest that P450/6.beta.B encodes an active P 450 form corresponding to CYP3A1 and P450PCN1 reported previously does not exist in rats. (c) 1999 Academic Press.

L22 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2002 ACS

1999:61536 Document No. 130:233905 Recombinant pinoresinol-lariciresinol reductases from western red cedar (Thuja plicata) catalyze opposite enantiospecific conversions. Fujita, Masayuki; Gang, David R.; Davin, Laurence B.; Lewis, Norman G. (Institute Biological Chemistry, Washington State University, Pullman, WA, 99164-6340, USA). Journal of Biological Chemistry, 274(2), 618-627 (English) 1999. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Although the heartwood of woody plants represents the main source of fiber and solid wood products, essentially nothing is known about how the biol. processes leading to its formation are initiated and regulated. Accordingly, a reverse transcription- ***polymerase*** ***chain*** ***reaction*** -guided cloning strategy was employed to obtain genes encoding pinoresinol-lariciresinol reductases from western red cedar (Thuja plicata) as a means to initiate the study of its heartwood formation. (+)-Pinoresinol-(+)-lariciresinol reductase from Forsythia intermedia was used as a template for primer construction for reverse transcription- ***polymerase*** ***chain*** ***reaction*** amplifications, which, when followed by homologous hybridization cloning, resulted in the isolation of two distinct classes of putative pinoresinol-lariciresinol reductase cDNA clones from western red cedar. A representative of each class was expressed as a fusion protein with .beta.-galactosidase and assayed for enzymic activity. Using both deuterated and radiolabeled (.+-.)-pinoresinols as substrates, it was established that each class of cDNA encoded a pinoresinol-lariciresinol reductase of different (opposite) enantiospecificity. Significantly, the protein from one class converted (+)-pinoresinol into (-)-secoisolariciresinol, whereas the other utilized the opposite (-)-enantiomer to give the corresponding (+)-form. This differential substrate specificity raises important questions about the role of each of these individual reductases in heartwood formation, such as whether they are expressed in different cells/tissues or at different stages during heartwood development.

L22 ANSWER 10 OF 20 CAPLUS COPYRIGHT 2002 ACS

1998:563672 Document No. 129:271665 Expression of CYP1B1 but not CYP1A1 by primary cultured human mammary stromal fibroblasts constitutively and in response to dioxin exposure: role of the Ah receptor. Eltom, Sakina E.; Larsen, Michele C.; Jefocate, Colin R. (Environmental Toxicology Center and The Department of Pharmacology, University of Wisconsin, Madison, WI, 53706, USA). Carcinogenesis, 19(8), 1437-1444 (English) 1998. CODEN: CRNGDP. ISSN: 0143-3334. Publisher: Oxford University Press.

AB The expression of CYP1B1 in human mammary fibroblasts (HMFs) was characterized as a potential modulator of their individual function as well as effects on adjacent mammary epithelia. We have used these characteristics to explore the diversity of fibroblast cells isolated from redn. mammoplasty patients and from different breast locations in breast cancer patients (tumors, peripheral to tumor and skin). These parameters have also been used to examine differences between two donors. The results have shown that while none of these HMFs expressed a detectable CYP1A1 protein basally or in response to TCDD, they all expressed CYP1B1 constitutively at similar levels (0.5-0.9 pmol/mg microsomal proteins) and they were induced by TCDD (up to 5-fold) consistent with mediation by the Ah receptor (AhR). DMBA metab. by HMFs exhibited high proportions of 5,6-, 10,11- and 3,4-dihydrodiols, a profile that is typical of human CYP1B1 ***regioselectivity*** . RT- ***PCR*** followed by Southern blot analyses demonstrated that CYP1B1 mRNA expression in HMFs parallels

levels of resp. microsomal proteins. The AhR is expressed in these HMFs as two cytosolic forms (.apprx.106 and 104 kDa) and a substantial proportion of the 104 kDa form was localized to the nucleus even prior to TCDD treatment. In all HMFs isolated directly from collagenase digested breast tissues the AhR is expressed at levels 10-fold lower than in breast epithelial cells. However, HMFs that were isolated after serial passaging of mammary epithelial cultures had shown much higher levels of the AhR expression and more dramatic TCDD-induced down-regulation (/>80% in 24 h) assocd. with more efficient nuclear translocation. These differences suggested the presence of two functionally distinct subtypes of HMFs: interstitial stromal fibroblasts that are readily released by collagenase digestion of breast tissues, and lobular stromal fibroblasts which are more tightly assocd. with the breast epithelia.

L22 ANSWER 11 OF 20 CAPLUS COPYRIGHT 2002 ACS

1998:559356 Document No. 129:312727 Changing substrate specificity and product pattern in adrenal cytochrome P-450-dependent steroid hydroxylases. Bottner, Benjamin; Cao, Peirang; Bernhardt, Rita (Max-Delbruck-Centrum Molekulare Medizin, Berlin, D-13122, Germany). Keio University Symposia for Life Science and Medicine, 1(Oxygen Homeostasis and Its Dynamics), 221-230 (English) 1998. CODEN: KUSMF9. Publisher: Springer-Verlag Tokyo.

AB In the human adrenal cortex, the mitochondrial cytochrome P 450-dependent 11.beta.-hydroxylase (CYP11B1) is responsible for the formation of cortisol, whereas the aldosterone synthase (CYP11B2), being 93% identical in its primary structure to CYP11B1, catalyzes the biosynthesis of aldosterone. Both proteins receive the necessary electrons for oxygen activation via an electron-supporting system consisting of adrenodoxin and adrenodoxin reductase. We have cloned the cDNA of human CYP11B1 and CYP11B2 using reverse transcriptase- ***polymerase*** ***chain*** ***reaction*** (RT- ***PCR***) methodol. Site-directed mutagenesis and computer modeling were used to investigate the mol. basis for the ***regioselectivity*** of steroid hydroxylation. Replacement of three amino acids of CYP11B2 by the corresponding residues of CYP11B1 were sufficient to increase cortisol formation from about 5% to 85% of the level obtained with CYP11B1. The aldosterone synthase activities of the mutant CYP11B2 proteins were suppressed to 10% of the CYP11B2 activity. When replacing these three residues of CYP11B1 by the amino acid found exclusively in CYP11B2, the mutant is able to form aldosterone. The capacity amts. to about 20% of that of CYP11B2. Taking into account that in human adrenals, CYP11B1 is considerably more strongly expressed than CYP11B2, a potential role of point mutations of CYP11B1 as a cause of hyperaldosteronism can be envisioned. As data from the literature suggest also that factors other than the primary structures of CYP11B1 and CYP11B2 could affect the amt. of aldosterone formed, we investigated whether the supply of electrons to bovine CYP11B0 would change the amt. of aldosterone prodn. For this purpose, mutant variants of adrenodoxin obtained by site-directed mutagenesis and possessing variable abilities to transfer electrons were studied. It could be demonstrated that mutants with an increased rate const. for the transfer of the first electron were able to increase the amt. of aldosterone produced by bovine CYP11B0.

L22 ANSWER 12 OF 20 CAPLUS COPYRIGHT 2002 ACS

1998:369903 Document No. 129:105449 Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic aromatic hydrocarbon metabolism. Larsen, Michele Campaigne; Angus, William G. R.; Brake, Paul B.; Eltom, Sakina E.; Sukow, Kristine A.; Jefcoate, Colin R. (Environmental Toxicology Center, University of Wisconsin, Madison, WI, 53706, USA). Cancer Research, 58(11), 2366-2374 (English) 1998. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

AB CYP1B1 and CYP1A1 expression and metab. of 7,12-dimethylbenz[a]anthracene (DMBA) have been characterized in early-passage human mammary epithelial cells (HMECs) isolated from redn. mammoplasty tissue of seven individual donors. The level of constitutive microsomal CYP1B1 protein expression was donor dependent (<0.01-1.4 pmol/mg microsomal protein). CYP1B1 expression was substantially induced by exposure of the cells to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to levels ranging from 2.3 to 16.6 pmol/mg among the seven donors. Extremely low, reproducible levels of constitutive CYP1A1 expression were detectable in three donors (0.03-0.16 pmol/mg microsomal protein). TCDD inductions were larger for

CYP1A1, as compared to CYP1B1, demonstrating substantial variability in the induced levels among the donors (0.8-16.5 pmol/mg). Northern and reverse transcriptase ***PCR*** analyses corroborate the donor-dependent differences in protein expression, whereby CYP1B1 mRNA (5.2 kb) was constitutively expressed and was highly induced by TCDD (33-fold). The contributions of CYP1B1 and CYP1A1 to the metab. of DMBA were analyzed using recombinant human CYP1B1 and CYP1A1, as refs., in conjunction with antibody-specific inhibition analyses (anti-CYP1B1 and anti-CYP1A1). Constitutive microsomal activity exhibited a profile of regioselective DMBA metab. that was characteristic of human CYP1B1 (increased proportions of 5,6- and 10,11-DMBA-dihydrodiols), which was inhibited by anti-CYP1B1 (84%) but not by anti-CYP1A1. TCDD-induced HMEC microsomal DMBA metab. generated the 8,9-dihydrodiol of DMBA as the predominant metabolite, with a ***regioselectivity*** similar to that of recombinant human CYP1A1, which was subsequently inhibited by anti-CYP1A1 (79%). A CYP1B1 contribution was indicated by the ***regioselectivity*** of residual metab. and by anti-CYP1B1 inhibition (25%). DMBA metab. analyses of one of three donors expressing measurable basal expression of CYP1A1 confirmed DMBA metab. levels equiv. to that from CYP1B1. The HMECs of all donors expressed similar, very high levels of the aryl hydrocarbon receptor and the aryl hydrocarbon nuclear translocator protein, suggesting that aryl hydrocarbon receptor and aryl hydrocarbon nuclear translocator protein expression are not responsible for differences in cytochrome P 450 expression. This study indicates that CYP1B1 is an important activator of polycyclic arom. hydrocarbons in the mammary gland when environmental chem. exposures minimally induce CYP1A1. Addnl., certain individuals express low levels of basal CYP1A1 in HMECs, representing a potential risk factor of mammary carcinogenesis through enhanced polycyclic arom. hydrocarbon bioactivation.

L22 ANSWER 13 OF 20 CAPLUS COPYRIGHT 2002 ACS

1998:269523 Document No. 129:24847 Expression and Characterization of Four Recombinant Human Dihydrodiol Dehydrogenase Isoforms: Oxidation of trans-7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene to the Activated o-Quinone Metabolite Benzo[a]pyrene-7,8-dione. Burczynski, Michael E.; Harvey, Ronald G.; Penning, Trevor M. (Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104-6084, USA). Biochemistry, 37(19), 6781-6790 (English) 1998. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The bioactivation of polycyclic arom. hydrocarbons (PAHs) to their ultimate carcinogenic forms proceeds via the formation of proximate carcinogen trans-dihydrodiols. Previous studies demonstrated that rat liver 3.alpha.-hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase (3.alpha.-HSD/DD), a member of the aldo-keto reductase (AKR) superfamily, oxidizes PAH trans-dihydrodiols to redox-cycling o-quinones. Multiple closely related AKRs exist in human liver; however, it is unclear which, if any, participate in PAH activation by catalyzing the NADP+-dependent oxidn. of PAH trans-dihydrodiols. In this study, cDNAs encoding four human DD isoforms were isolated from HepG2 cells using isoform-selective RT- ***PCR*** . The recombinant proteins were overexpressed in Escherichia coli, purified to homogeneity, and kinetically characterized. Calcn. of KM and kcat values of each isoform for model substrates revealed that they possessed enzymic activities assigned to native human liver DD1, DD2, DD4, and type 2 3.alpha.-HSD (DDX) proteins. The ability of human DDs to oxidize the potent proximate carcinogen (.+-.)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP-diol) was then examd. A reverse phase HPLC radiochem. assay demonstrated that all four isoforms oxidize (.+-.)-BP-diol in the following rank order: DD2 > DD1 > DD4 > DDX. Each DD consumed the entire racemic BP-diol mixt., indicating that both the minor (+)-S,S- and major (-)-R,R-stereoisomers formed in vivo are substrates. First-order decay plots showed that DD1 and DD2 displayed preferences for one of the stereoisomers, and CD spectroscopy indicated that this isomer was the (+)-7S,8S-enantiomer. The products of these reactions were trapped as either glycine or thiol ether conjugates of benzo[a]pyrene-7,8-dione (BPQ), indicating that the initial oxidn. product was the reactive BPQ. Thus, human liver possesses multiple AKRs which contribute to PAH activation by catalyzing the NADP+-dependent oxidn. of PAH trans-dihydrodiols to redox-active o-quinones.

L22 ANSWER 14 OF 20 CAPLUS COPYRIGHT 2002 ACS

1997:705709 Document No. 127:341361 Genetic association between sensitivity

to warfarin and expression of CYP2C9*3. Steward, Daniel J.; Haining, Robert L.; Henne, Kirk R.; Davis, George; Rushmore, Thomas H.; Trager, William F.; Rettie, Allan E. (Department of Medicinal Chemistry, University of Washington, Seattle, WA, 98195, USA). Pharmacogenetics, 7(5), 361-367 (English) 1997. CODEN: PHMCEE. ISSN: 0960-314X. Publisher: Chapman & Hall.

AB Cytochrome P 450 2C9 (CYP2C9) is largely responsible for terminating the anticoagulant effect of racemic warfarin via hydroxylation of the pharmacol. more potent S-enantiomer to inactive metabolites. Mutations in the CYP2C9 gene result in the expression of three allelic variants, CYP2C9*1, CYP2C9*2 and CYP2C9*3. Both CYP2C9*2 and CYP2C9*3 exhibit altered catalytic properties in vitro relative to the wild-type enzyme. In the present study, a patient was genotyped who had proven unusually sensitive to warfarin therapy and could tolerate no more than 0.5 mg of the racemic drug/day. ***PCR*** -amplification of exons 3 and 7 of the CYP2C9 gene, followed by restriction digest or sequence anal., showed that this individual was homozygous for CYP2C9*3. In addn., patient plasma warfarin enantiomer ratios and urinary 7-hydroxywarfarin enantiomer ratios were detd. by chiral-phase high performance liq. chromatog. to investigate whether either parameter might be of diagnostic value in place of a genotypic test. Control patients receiving 4-8 mg warfarin/day exhibited plasma S:R ratios of 0.50:1, whereas the patient on very low-dose warfarin exhibited an S:R ratio of 3.9:1. In contrast, the urinary 7-hydroxywarfarin S:R ratio of 4:1 showed the same ***stereoselectivity*** as that reported for control patients. Therefore, expression of CYP2C9*3 is assocd. with diminished clearance of S-warfarin and a dangerously exacerbated therapeutic response to normal doses of the racemic drug. Anal. of the plasma S:R warfarin ratio may serve as a useful alternative test to genotyping for this genetic defect.

L22 ANSWER 15 OF 20 CAPLUS COPYRIGHT 2002 ACS

1996:697386 Document No. 125:318456 D2-like dopamine receptors are not detectable on human peripheral blood lymphocytes. Vile, John M.; Strange, Philip G. (Research School Biosciences, University, Canterbury, UK). Biological Psychiatry, 40(9), 881-885 (English) 1996. CODEN: BIPCBF. ISSN: 0006-3223. Publisher: Elsevier.

AB The binding of [3H]-nemonapride to human peripheral blood lymphocytes (PBL) was studied using various competing ligands specific for D2-like dopamine receptors. There is no detectable ***stereoselectivity*** for the stereoisomers of butaclamol, and competitions with haloperidol and sulpiride also show no evidence of specific binding to D2-like dopamine receptors. RT- ***PCR*** of RNA from human lymphocytes showed that there is no detectable D2 mRNA (even with nested ***PCR***). D3 mRNA was, however, detectable by RT- ***PCR*** , but only at low levels that could not be detected by Northern blots of PBL total RNA.

L22 ANSWER 16 OF 20 CAPLUS COPYRIGHT 2002 ACS

1996:256014 Document No. 124:336629 Molecular cloning and characterization of a novel human diacylglycerol kinase .zeta.. Bunting, Michaeline; Tang, Wen; Zimmerman, Guy A.; McIntyre, Thomas M.; Prescott, Stephen M. (Eccles Program Human Mol. Biol. Genet., Univ. Utah, Salt Lake City, UT, 84112, USA). Journal of Biological Chemistry, 271(17), 10230-6 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Diacylglycerol (DAG) occupies a central position in the synthesis of complex lipids and also has important signaling roles. For example, DAG is an allosteric regulator of protein kinase C, and the cellular levels of DAG may influence a variety of processes including growth and differentiation. The authors previously demonstrated that human endothelial cells derived from umbilical vein express growth-dependent changes in their basal levels of diacylglycerol and diacylglycerol kinase activity (Whatley, R. E., Stroud, E. D., Bunting, M., Zimmerman, G. A., McIntyre, T. M., and Prescott, S. M. (1993) J. Biol. Chem. 268, 16130-16138). To further explore the role of diacylglycerol metab. in endothelial responses, the authors used a degenerate reverse transcription- ***polymerase*** ***chain*** ***reaction*** method to identify diacylglycerol kinase isoenzymes expressed by human endothelial cells. They report the isolation of a 3.5-kilobase cDNA encoding a novel diacylglycerol kinase (hDGK.zeta.) with a predicted mol. mass of 103.9 kDa. Human DGK.zeta. contains two zinc fingers, an ATP binding site, and four ankyrin repeats near the carboxyl terminus. A

unique feature, as compared with other diacylglycerol kinases, is the presence of a sequence homologous to the MARCKS phosphorylation site domain. From Northern blot anal. of multiple tissues, it was obsd. that hDGK.zeta. mRNA is expressed at highest levels in brain. COS-7 cells transfected with the hDGK.zeta. and cDNA express 117-kDa and 114-kDa proteins that react specifically with an antibody to a peptide derived from a unique sequence in hDGK.zeta.. The transfected cells also express increased diacylglycerol kinase activity, which is not altered in the presence of R59949, an inhibitor of human platelet DGK activity. The hDGK.zeta. displays ***stereoselectivity*** for 1,2-diacylglycerol species in comparison to 1,3-diacylglycerol, but does not exhibit any specificity for mol. species of long chain diacylglycerols.

L22 ANSWER 17 OF 20 CAPLUS COPYRIGHT 2002 ACS

1996:234295 Document No. 124:279823 Localization and characterization of atypical .beta.-adrenoceptors in skeletal muscle and gut. Summers, R. J.; Russell, F. D.; Roberts, S. J.; Bonazzi, V. R.; Sharkey, A.; Evans, B. A.; Molenaar, P. (Dep. Pharmacology, Univ. Melbourne, Parkville, 3052, Australia). Pharmacology Communications, 6(1-3), 237-52 (English) 1995. CODEN: PCMME9. ISSN: 1060-4456. Publisher: Harwood.

AB (-)-[125I]-CYP binds to high affinity and low affinity sites in rat soleus muscle (RS) and human colon (HC). High affinity sites (KD 30.5 and 12 pM resp.) correspond to conventional .beta.-adrenoceptors while low affinity (KD 522.5 and 691 pM) sites have pharmacol. and regulatory properties appropriate for atypical .beta.-adrenoceptors. Competition for low affinity sites in RS and HC showed that antagonists competed for binding with a low degree of ***stereoselectivity*** (***stereoselectivity*** indexes tertatolol HC 9.3, RS 2.6; alprenolol HC 6.8, RS 3.1). Comparison of the sites in RS and HC showed them to be similar to each other and to atypical .beta.-adrenoceptors in gastrointestinal studies but clearly different from .beta.1- or .beta.2-adrenoceptors. Autoradiog. studies of atypical .beta.-adrenoceptors in rat hind limb muscle showed localization to soleus and other oxidative muscle fibers and colocalization of .beta.2-adrenoceptors. In HC autoradiog. studies showed high concns. of atypical .beta.-adrenoceptors in mucosa, submucosal arteries and veins, myenteric plexus and longitudinal smooth muscle. In contrast, .beta.1-adrenoceptors were found in muscularis mucosae, submucosal arteries and longitudinal smooth muscle and .beta.2-adrenoceptors at low levels in mucosa, submucosal and in localized areas in smooth muscle. Mol. studies detected .beta.3-mRNA in soleus muscle by RT/ ***PCR*** and localized it to mucosa by in situ hybridization in human colon. These studies provide further evidence for the presence of .beta.3-adrenoceptors in rat soleus and human colon localized to areas where they are likely to have metabolic, secretory or absorptive roles.

L22 ANSWER 18 OF 20 CAPLUS COPYRIGHT 2002 ACS

1996:118153 Document No. 124:195303 Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. Furuya, Hirokazu; Fernandez-Salguero, Pedro; Gregory, Wendy; Taber, Heather; Steward, Annette; Gonzalez, Frank J.; Idle, Jeffrey R. (Laboratory of Molecular Carcinogenesis, NCI, Bethesda, MD, 20892, USA). Pharmacogenetics, 5(6), 389-92 (English) 1995. CODEN: PHMCEE. ISSN: 0960-314X. Publisher: Chapman & Hall.

AB A single amino acid substitution of cysteine for arginine at position 144 in the CYP2C9 protein, arising from a base substitution, gives rise to an allelic variant of CYP2C9 termed R144C. It has been found that the mutation markedly affected neither the regio- nor the ***stereoselectivity*** of warfarin hydroxylation. Expression of the variant cDNA revealed a profound effect of this single amino acid change. It should be expected therefore that the R144C allele would affect the metab. of (S)-warfarin in vivo. In order to examine this proposition, the authors have investigated the CYP2C9 genotype by ***PCR*** in a series of patients attending an anticoagulation clinic for routine monitoring and related these findings to their individual warfarin dose requirement for stable anticoagulation.

L22 ANSWER 19 OF 20 CAPLUS COPYRIGHT 2002 ACS

1995:495569 Document No. 122:285118 The molecular biology of the flavin-containing monooxygenases of man. Phillips, Ian R.; Dolphin, Colin T.; Clair, Philippe; Hadley, Mark R.; Hutt, Andrew J.; McCombie, Richard

R.; Smith, Robert L.; Shephard, Elizabeth A. (Department of Biochemistry, Queen Mary and Westfield College, University of London, Mile End Road, London, E1 4NS, UK). Chemico-Biological Interactions, 96(1), 17-32 (English) 1995. CODEN: CBINA8. ISSN: 0009-2797. Publisher: Elsevier.

AB A review, with 36 refs. CDNA clones encoding five distinct members of the FMO family of man (FMOs 1, 2, 3, 4 and 5) were isolated by a combination of library screening and reverse transcription- ***polymerase*** ***chain*** ***reaction*** techniques. The deduced amino acid sequences of the human FMOs have 82-87% identity with their known orthologues in other mammals but only 51-57% similarity to each other. The hydropathy profiles of the proteins are very similar. From the calcd. rate of evolution of FMOs (a 1% change in sequence per 6 million years) it would appear that individual members of the FMO gene family arose by duplication of a common ancestral gene some 250-300 million years ago. Each of the FMO genes was mapped by the ***polymerase*** ***chain*** ***reaction*** to the long arm of human chromosome 1. The localization of the FMO1 gene was further refined to 1q23 - q25 by in situ hybridization of human metaphase chromosomes. RNase protection assays demonstrated that in man each FMO gene displays a distinct developmental and tissue-specific pattern of expression. In the adult, FMO1 is expressed in kidney but not in liver, whereas in the fetus its mRNA is abundant in both organs. FMO3 expression is essentially restricted to the liver in the adult and the mRNA is either absent, or present in low amts., in fetal tissues. FMO4 is expressed more constitutively. Human FMO1 and FMO3 cDNAs were functionally expressed in prokaryotic and eukaryotic cells. FMO1 and FMO3, expressed in either system, displayed product ***stereoselectivity*** in their catalysis of the N-oxidn. of the pro-chiral tertiary amines, N-ethyl-N- methylaniline (EMA) and pargyline. Both enzymes were stereoselective with respect to the prodn. of the (-)-S-enantiomer of EMA N-oxide. But in the case of pargyline, the enzymes displayed opposite ***stereoselectivity*** , FMO1 producing solely the (+)-enantiomer and FMO3 predominantly the (-)-enantiomer of the N-oxide.

L22 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2002 ACS

1993:32842 Document No. 118:32842 Identification of a functionally relevant cannabinoid receptor on mouse spleen cells that is involved in cannabinoid-mediated immune modulation. Kaminski, Norbert E.; Abood, Mary E.; Kessler, Fay K.; Martin, Billy R.; Schatz, Anthony R. (Med. Coll. Virginia, Virginia Commonw. Univ., Richmond, VA, 23298, USA). Mol. Pharmacol., 42(5), 736-42 (English) 1992. CODEN: MOPMA3. ISSN: 0026-895X.

AB Extensive behavioral and biochem. characterization of cannabinoid-mediated effects on the central nervous system has revealed at least three lines of evidence supporting the role of a putative guanine nucleotide-binding protein-coupled cannabinoid receptor for cannabimimetic effects: (i) ***stereoselectivity*** , (ii) inhibition of the adenylate cyclase/cAMP second messenger system, and (iii) radioligand-binding studies with the synthetic cannabinoid [3H]CP-55940 indicating a high degree of specific binding to brain tissue prepns. Based on recent findings from our lab. demonstrating that .DELTA.9-tetrahydrocannabinol markedly inhibited forskolin-stimulated cAMP accumulation in mouse spleen cells, the presence of a guanine nucleotide-binding protein-coupled cannabinoid receptor assocd. with mouse spleen cells and its functional role in immune modulation were investigated. In the present studies, stereoselective immune modulation was obsd. with the synthetic bicyclic cannabinoid (-)-CP-55940 vs. (+)-CP-56667 and with 11-OH-.DELTA.8-tetrahydrocannabinoldimethylheptyl, (-)-HU-210 vs. (+)-HU-211. In both cases, the (-)-enantiomer demonstrated greater immunoinhibitory potency than the (+)-isomer, as measured by the in vitro sheep red blood cell antibody-forming cell response. Radioligand binding studies produced a satn. isotherm exhibiting approx. 45-65% specific binding to mouse spleen cells. Scatchard anal. demonstrated a single binding site on spleen cells, possessing a Kd of 910 pm and a Bmax of approx. 1000 receptors/spleen cell. RNA ***polymerase*** ***chain*** ***reaction*** of isolated splenic RNA using specific primers for the cannabinoid receptor resulted in the amplification of a 854-kilobase predicted product that hybridized with cannabinoid receptor cDNA, demonstrating the presence of cannabinoid receptor mRNA in mouse spleen. Together, these findings strongly support the role of a cannabinoid receptor in immune modulation by cannabimimetic agents.

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=> S L7 AND (L8,L9,L10,L11,L12,L13,L14)
L23         573 L7 AND ((L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14))

=> S L23 NOT L22
L24         570 L23 NOT L22

=> S CONCENTRATION
         120727 CONCENTRATION
          58794 CONCENTRATIONS
         178444 CONCENTRATION
                (CONCENTRATION OR CONCENTRATIONS)
        1602216 CONCN
         984016 CONCNS
        2227175 CONCN
                (CONCN OR CONCNS)
L25     2265745 CONCENTRATION
                (CONCENTRATION OR CONCN)
 75% OF LIMIT FOR TOTAL ANSWERS REACHED

=> S L25 (4A)(L8,L9)
L26       74224 L25 (4A)((L8 OR L9))

=> S L24 AND L26
L27          16 L24 AND L26

=> S 1-16 CBIB ABS\
        7273370 1
         676009 16
              9 CBIB
         201699 ABS
              4 ABSES
         201703 ABS
                (ABS OR ABSES)
L28           0 1-16 CBIB ABS\
                (1(W)16(W)CBIB(W)ABS)

=> S 1-16 CBIB ABS
        7273370 1
         676009 16
              9 CBIB
         201699 ABS
              4 ABSES
         201703 ABS
                (ABS OR ABSES)
L29           0 1-16 CBIB ABS
                (1(W)16(W)CBIB(W)ABS)

=> D L27 1-16 CBIB ABS

L27  ANSWER 1 OF 16  CAPLUS  COPYRIGHT 2002 ACS
2002:458546  Expression of Cardamom mosaic virus coat protein in Escherichia
     coli and its assembly into filamentous aggregates.  Jacob, Thomas; Usha,
     R. (School of Biotechnology, Department of Plant Biotechnology, Madurai
     Kamaraj University, Madurai, 625021, India).  Virus Research, 86(1-2),
     133-141 (English) 2002.  CODEN: VIREDF.  ISSN: 0168-1702.  Publisher:
     Elsevier Science Ltd..
AB   Cardamom mosaic virus (CdMV), a member of the genus Macluravirus of
     Potyviridae, causes a mosaic disease in cardamom.  A polyclonal antiserum
     was raised against the purified virus and IgG was prepd.  Electron
     microscopic studies on the purified virus showed flexuous filamentous
     particles of .apprx.800 nm in length, typical of members of Potyviridae.
     The coat protein (CP) encoding sequence of the virus was expressed in
     Escherichia coli and the protein purified by affinity chromatog. under
     denaturing conditions.  The viral nature of the expressed CP was confirmed
     by pos. reaction with anti CdMV IgG in a Western blot.  The expressed CP
     aggregated irreversibly upon renaturation at   ***concns***  . above 0.07
       ***mg***  /mL.  The expression of the CP led to the formation of
     filamentous aggregates in E. coli as obsd. by immuno-gold electron
     microscopy.  The filamentous aggregates were of 100-150 nm in length.
     Immuno-capture RT-  ***PCR***   confirmed the absence of coat protein mRNA
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in the filamentous aggregates. Deletion mutations, which were expected to inhibit virus assembly, were introduced in the core ***region*** of the coat protein. However, these mutations did not improve the soly. of the CP in non-denaturing buffers.

L27 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2002 ACS

2002:263197 Document No. 136:351521 Rhizobium leguminosarum bv. viciae populations in soils with increasing heavy metal contamination: abundance, plasmid profiles, diversity and metal tolerance. Lakzian, Amir; Murphy, Phillip; Turner, Andrew; Beynon, Jim L.; Giller, Ken E. (Department of Biological Sciences, Wye College, University of London, Wye, Ashford, Kent, TN25 5AH, UK). Soil Biology & Biochemistry, 34(4), 519-529 (English) 2002. CODEN: SBIOAH. ISSN: 0038-0717. Publisher: Elsevier Science Ltd..

AB Populations of Rhizobium leguminosarum bv. viciae were investigated from plots of a long-term sewage sludge expt. in Braunschweig, Germany, which represented a gradient of increasing metal contamination. The no. of R. leguminosarum bv. viciae decreased from 105 cells g-1 soil in uncontaminated plots to between 7 and 102 cells g-1 soil with increasing Zn ***concn*** . (from 50 to 400 ***mg*** kg-1). Rhizobia were isolated from nodules of Vicia hirsuta inoculated with dilns. of soil from seven of the plots (.apprx.50 isolates per plot). The rhizobial isolates had between three and nine plasmids which varied in size from approx. 100 to 850 kb. Although a total of 49 plasmid profile groups were identified, ***PCR*** -RFLP anal. using primers which amplified an intergenic spacer (ITS) ***region*** between the 16S and 23S rRNA genes revealed only 20 groups. Ten ITS groups were found among the isolates from the uncontaminated control plot but only two groups were found in the most contaminated plot, and six to eight groups in the plots with intermediate metal contamination. Nos. of plasmid profile groups increased with moderate metal-contamination but were strongly reduced when total Zn ***concns*** . exceeded 300 ***mg*** kg-1. Isolates from the less contaminated plots had only three to five plasmids whereas isolates with seven to nine plasmids were abundant in the plots with metal ***concns*** . of 200 ***mg*** kg-1 or more. Whereas plasmid profiles indicated considerable changes in strains with increasing metal contamination, one ITS group (group 1) was present in all plots. Isolates from ITS group 1 contained only three to four plasmids in the control plots but those from the most contaminated plots had eight to nine plasmids. There was a marked increase in metal tolerance of isolates belonging to ITS group 1 as metal contamination increased, which was assocd. with the increase in the no. of plasmids carried. However, another ITS group (group 2), which had only three large plasmids was present only in the most contaminated plot. Isolates from this group had less tolerance to Zn than many isolates from the uncontaminated plots. Possible mechanisms for the survival of these isolates in the metal contaminated soils are discussed.

L27 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2002 ACS

2002:227324 Document No. 136:352445 Biodegradation of .gamma.-hexachlorocyclohexane (Lindane) and .alpha.-hexachlorocyclohexane in water and a soil slurry by a Pandoraea species. Okeke, Benedict C.; Siddique, Tariq; Arbestain, Marta Camps; Frankenberger, William T. (Department of Environmental Sciences, University of California, Riverside, CA, 92521, USA). Journal of Agricultural and Food Chemistry, 50(9), 2548-2555 (English) 2002. CODEN: JAFCAU. ISSN: 0021-8561. Publisher: American Chemical Society.

AB Isomers of 1,2,3,4,5,6-hexachlorocyclohexane (HCH) were some of the most widely used pesticides. Despite redn. in their prodn. and use, HCH isomers present a serious environmental hazard. In this study, 2 bacterial isolates (LIN-1 and LIN-3) that can grow on .gamma.-HCH as a sole source of C and energy were isolated from an enrichment culture. In liq. cultures of LIN-1 and LIN-3, 25.0 and 45.5% removal of .gamma.-HCH, resp., were achieved in 2 wk. LIN-3 was identified as Pandoraea sp. by 16S rRNA gene sequence anal. (99% identity). Pandoraea sp. substantially degraded both .gamma.- and .alpha.-HCH isomers at ***concns*** . of 10-200 ***mg*** /L in liq. cultures. After 8 wk of incubation in liq. culture, 89.9 and 93.3% of the .gamma.- and .alpha.-HCH isomers declined, resp., at an initial ***concn*** . of 150 ***mg*** /L. In soil slurry cultures of Pandoraea sp., simulating a soil slurry phase bioremediation treatment, substantial decreases in the levels of the HCH

isomers were obsd. at ***concns*** . of 50-200 ***mg*** /L. After 9 wk, 59.6 and 53.3% biodegrdns. of .gamma.- and .alpha.-HCH isomers, resp., were achieved at 150 ***mg*** /L. Using 2 23-mer oligonucleotide primers targeting the 330-bp ***region*** of the 16S rRNA gene of Pandoraea sp., an approx. 330 bp ***PCR*** product was successfully amplified from DNA templates prepd. from bacterial colonies and soil slurry culture. This system provides a direct and rapid ***PCR*** -based mol. tool for tracking Pandoraea sp. strain LIN-3 in water and soils. These results have implied implications for the treatment of soils and water contaminated with HCH isomers.

L27 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2002 ACS

2002:172088 Document No. 136:227877 ***PCR*** primers for construction of transcription template for dilution batch-type cell-free protein synthesis system. Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio (Wakenyaku Co., Ltd., Japan). PCT Int. Appl. WO 2002018586 A1 20020307, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2001-JP7357 20010828. PRIORITY: JP 2000-261638 20000830; JP 2001-58404 20010302.

AB Primer sequences for construction of templates for a cell-free protein synthesis system using wheat germ ext. are described. The 3'-terminal ***PCR*** primer contains sequences complementary to the sequence between the transcriptional termination sequence of a reporter gene (for example, a drug-resistance gene) of the vector and Ori. The 5'-terminal primer contains sequences complementary to part of the promoter sequence. Those two types of primers satisfy the requirement of not priming the transcription from a DNA constructed by using only of those primers alone. One of those having a sequence complementary to part of the RNA polymerase recognition site from the 5'-terminus of the promoter and another having a sequence complementary to part of the RNA polymerase recognition site from the 3'-terminus of the promoter are provided as primers for 5'-terminal ***PCR*** . GA or GAA sequences is ligated to those sequences, and further downstream, transcription initiation codon ATG, part of the target gene ORF. A histidine tag, glutathione-S-transferase (GST), or myb tag or epitope prepn. sequence may also be used. The reaction mixt. is to dild. achieve the optimal ***magnesium*** ***concn*** . Cell-free protein synthesis using a modified mRNA having a tobacco mosaic virus (TMV) .OMEGA. sequence ligated to the 5'-end and an untranslated ***region*** (UTR) ligated to the 3'-end, is described.

L27 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2002 ACS

2002:121894 Document No. 137:150676 Multiplex ***polymerase*** ***chain*** ***reaction*** : A practical approach. Markoulatos, P.; Siafakas, N.; Moncany, M. (Virology Department, Hellenic Pasteur Institute, Athens, 115 21, Greece). Journal of Clinical Laboratory Analysis, 16(1), 47-51 (English) 2002. CODEN: JCANEM. ISSN: 0887-8013. Publisher: Wiley-Liss, Inc..

AB A review. Considerable time and effort can be saved by simultaneously amplifying multiple sequences in a single reaction, a process referred to as multiplex ***polymerase*** ***chain*** ***reaction*** (***PCR***). Multiplex ***PCR*** requires that primers lead to amplification of unique ***regions*** of DNA, both in individual pairs and in combinations of many primers, under a single set of reaction conditions. In addn., methods must be available for the anal. of each individual amplification product from the mixt. of all the products. Multiplex ***PCR*** is becoming a rapid and convenient screening assay in both the clin. and the research lab. The development of an efficient multiplex ***PCR*** usually requires strategic planning and multiple attempts to optimize reaction conditions. For a successful multiplex ***PCR*** assay, the relative concn. of the primers, concn. of the ***PCR*** buffer, balance between the ***magnesium*** chloride and deoxynucleotide ***concns*** ., cycling temps., and amt. of template DNA and Taq DNA polymerase are important. An optimal combination of annealing temp. and buffer concn. is essential in multiplex ***PCR***

to obtain highly specific amplification products. ***Magnesium*** chloride ***concn*** . needs only to be proportional to the amt. of dNTP, while adjusting primer concn. for each target sequence is also essential. The list of various factors that can influence the reaction is by no means complete. Optimization of the parameters discussed in the present review should provide a practical approach toward resolving the common problems encountered in multiplex ***PCR*** (such as spurious amplification products, uneven or no amplification of some target sequences, and difficulties in reproducing some results). Thorough evaluation and validation of new multiplex ***PCR*** procedures is essential. The sensitivity and specificity must be thoroughly evaluated using standardized purified nucleic acids. Where available, full use should be made of external and internal quality controls, which must be rigorously applied. As the no. of microbial agents detectable by ***PCR*** increases, it will become highly desirable for practical purposes to achieve simultaneous detection of multiple agents that cause similar or identical clin. syndromes and/or share similar epidemiol. features.

L27 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2002 ACS

2001:671324 Document No. 135:262342 Simultaneous spectrophotometric determination of calcium and ***magnesium*** in dialysis fluids using multivariate calibration methods. Kargosha, Kazem; Sarrafi, Amir H. M. (Chemistry and Chemical Engineering Research Center of Iran, Tehran, Iran). Analytical Letters, 34(10), 1781-1793 (English) 2001. CODEN: ANALBP. ISSN: 0003-2719. Publisher: Marcel Dekker, Inc..

AB A method for simultaneous spectrophotometric detn. of calcium and ***magnesium*** in dialysis fluids using multivariate calibration methods is proposed. The method is based on the development of the reaction between the analytes and Eriochrome Black T at pH 10.1. The parameters of chemometric procedure such as the spectral ***region*** and the no. of factors were optimized. Three more convenient models of ***PCR*** , PLS-1 and PLS-2 were applied and the results were compared. Synthetic solns. contg. different ***concns*** . of calcium and ***magnesium*** were used to check the prediction ability of these chemometric methods. The optimum method was applied to the anal. of com. dialysis fluids and the accuracy of proposed procedure was compared with that obtained by the USP ref. method. These measurements carried out with good precision and relative std. deviation of real samples anal. (n = 3) are <1.85%. Also the presence of the other components frequently added to dosage forms caused no interference effect in the proposed method.

L27 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2002 ACS

2001:64186 Document No. 134:126760 Method for amplifying low abundance nucleic acid sequences. Richardson, Peter; Cox, Peter (Cambridge University Technical Services, UK). PCT Int. Appl. WO 2001006004 A2 20010125, 120 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-EP6887 20000719. PRIORITY: US 1999-PV144666 19990719.

AB The present invention relates to methods as well as to nucleic acid primers and kits contg. the same for performing efficiently an amplification of nucleic acid sequences from a sample, particularly of nucleic acid sequences that are initially poorly represented in said sample. First, the mRNA species are reverse transcribed using a first heeled primer population to provide first-strand cDNA sequences, where the heel refers to a non-hybridizing ***region*** in the primmer. Second cDNA strands are synthesized from the first-strand cDNA using a second heeled primer population. The first and second cDNA strands are amplified over a no. of amplification cycles with the aid of a thermoresistant DNA polymerase under low stringency hybridization conditions with: (1) a primer comprising at least a portion of the heel sequence of the first heeled primer, and (2) a second primer comprising at least a portion of the heel sequence of the second heeled primer. Particularly preferred variable sequences in the second primers are: 5'-CGAGA-3', 5'-CGACA-3', 5'-CGTAC-3', and 5'-ATGCG-3', and a preferred heel sequence of the second

heeled primer population comprises 5'-CTGCATCTATCTAATGCTCC-3' or 5'-CTGCATCTATCTAGTACGCGT-3'. Higher stringency hybridization conditions may be used to prevent the generation of bridged nucleic acids; bridges are cleaved by using primers contg. a rare restriction cleavage site in its heel sequence. This method increases the proportion of high-mol.-wt. DNA mols, and uses or analyzes specific sequences present in the high-mol.-wt. DNA mols. When applied to mRNA samples obtained from cholinergic neurons, the invention detects the expression of low-abundance A1 receptor mRNA at levels 50-fold lower than those possible using previous methods. In addn., when applying the method of the invention to 2.5 ng of total RNA (equiv. to that contained in .apprx.250 cells), specific gene sequences could be detected using one millionth of the final product.

L27 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2002 ACS

1999:635032 Document No. 132:147266 An unexpected product from ***polymerase*** ***chain*** ***reaction*** -mediated site-directed mutagenesis due to misalignment of the mismatched primer. Baker, Darren W.; Rothberg, Paul G. (Molecular Genetics Laboratory, Children's Mercy Hospital, Kansas City, MO, 64108, USA). Molecular Diagnosis, 3(3), 157-161 (English) 1998. CODEN: MDIAFU. ISSN: 1084-8592. Publisher: Churchill Livingstone.

AB Background: The I1307K (T3920.fwdarw.A) variant of the APC gene has been identified as a potential risk factor for colorectal cancer and is present in 6% of Ashkenazi Jews. Screening for this mutation may allow identification of people at elevated risk who would benefit from increased surveillance. Methods and Results: We designed an assay to detect the T3920.fwdarw.A allele using a primer mismatched at the 3' terminal nucleotide in the ***polymerase*** ***chain*** ***reaction*** (***PCR***) to generate a recognition site for the restriction enzyme Mse I. After optimization of the ***PCR*** for ***magnesium*** ion ***concn*** . and annealing temp., the amplicon did not cut completely with the restriction enzyme in each of four tested DNAs. Sequence anal. of the ***PCR*** product that was resistant to digestion revealed that the T3920.fwdarw.A variant was not present. The artifact was caused by a single nucleotide loop-out in the genomic DNA template under the 3' ***region*** of the primer, which allowed the 3' terminal base of the primer to hybridize properly. As a result, the mismatched primer created a modified product different from that originally planned. At a ***magnesium*** ion ***concn*** . below the optimum for product yield, most of the product was digested by Mse I. Sequence anal. showed that, under these conditions, the intended product was produced. Conclusions: Mismatched primers can produce unintended products in a ***PCR*** due to looping out of a nucleotide in the template or the primer. The ***magnesium*** ion ***concn*** . can influence the sequence and amt. of the product.

L27 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2002 ACS

1998:643774 Document No. 130:49704 High-level chloramphenicol resistance in Neisseria meningitidis. Galimand, Marc; Gerbaud, Guy; Guibourdenche, Martine; Riou, Jean-Yves; Courvalin, Patrice (National Reference Center for Antibiotics and the Unite des Agents Antibacteriens, National Reference Center for Antibiotics and the Unite des Agents Antibacteriens, Institut Pasteur, Paris, 75724, Fr.). New England Journal of Medicine, 339(13), 868-874 (English) 1998. CODEN: NEJMAG. ISSN: 0028-4793. Publisher: Massachusetts Medical Society.

AB Neisseria meningitidis is nearly always susceptible to the penicillins, the cephalosporins, and chloramphenicol. Between 1987 and 1996, however, chloramphenicol-resistant strains were isolated from 11 patients in Vietnam and 1 in France. The minimal inhibitory concn. of chloramphenicol was detd. for the 12 isolates. The isolates were analyzed by monoclonal-antibody-based serotyping and subtyping, pulsed-field gel electrophoresis, and multilocus enzyme electrophoresis. Bacterial DNA was analyzed by hybridization, the ***polymerase*** ***chain*** ***reaction*** , and sequencing to identify the resistance gene and det. the origin of the resistance. The isolates were resistant to chloramphenicol (minimal inhibitory ***concn*** ., .gtoreq.64 ***mg*** per L) and produced an active chloramphenicol acetyltransferase. All 12 strains belonged to serogroup B but had a high degree of diversity, and 10 could not be typed with the use of monoclonal antibodies. The nucleotide sequence of the resistance gene and the

flanking ***regions*** was identical to that of an internal portion of transposon Tn4451 that carries the catP gene in Clostridium perfringens. Moreover, this gene was located in the same genomic site in the chloramphenicol-resistant isolates. The high-level chloramphenicol resistance that we describe in N. meningitidis isolates is of great concern, since in developing countries, chloramphenicol given i.m. is the std. therapy for meningococcal meningitis. The resistance to chloramphenicol is due to the presence of the catP gene on a truncated transposon that has lost mobility because of internal deletions, and the transformation of genetic material between strains of N. meningitidis probably played an important part in the dissemination of the gene.

L27 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2002 ACS

1998:11091 Document No. 128:123958 Identification of motilin mRNA in the brain of man and rabbit. Conservation of polymorphism of the motilin gene across species. Deportere, I.; De Clercq, P.; Svoboda, M.; Bare, L.; Peeters, T. L. (Gut Hormone Lab., Katholieke Univ. Leuven, Louvain, Belg.). Peptides (New York), 18(10), 1497-1503 (English) 1997. CODEN: PPTDD5. ISSN: 0196-9781. Publisher: Elsevier Science Inc..

AB The data regarding the identity of motilin-like immunoreactivity in the central nervous system are controversial. The aim of the present study was to clarify whether motilin mRNA is present in the brain of rabbit and man. Total RNA, prepd. from several ***regions*** of the rabbit brain, was subjected to RT- ***PCR*** aimed at amplifying a 294 bp cDNA fragment of the rabbit motilin precursor. The amplified product was subcloned and sequenced. The sequence showed 7 differences compared to the one reported for the duodenal precursor. However, the duodenal precursor from the rabbit used in the present study revealed identical substitutions. One of these, involving amino acid -11 of the signal peptide, was shown to be due to gene polymorphism, as has also been described at this site in man. By RIA the highest ***concn*** . of motilin (fmol/ ***mg*** protein) was detected in the hippocampus, the lowest in the telencephalon. Using a similar approach, but starting from com. human brain mRNA, the sequence of a comparable cDNA fragment of the human brain motilin precursor was obtained. Its sequence was identical with the one published for the human intestinal precursor. The authors' study demonstrates that motilin mRNA is present in the brain of man and rabbit. Together with the authors' recent findings of central motilin receptors, they suggest a central role for motilin.

L27 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2002 ACS

1997:399277 Document No. 127:76821 Mapping of 22 expressed sequence tags isolated from a porcine small intestine cDNA library. Joergensen, Claus B.; Winterooe, Anne Katrine; Yerle, Martine; Fredholm, Merete (Division of Animal Genetics, Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen, 1870, Den.). Mammalian Genome, 8(6), 423-427 (English) 1997. CODEN: MAMGEC. ISSN: 0938-8990. Publisher: Springer.

AB Complementary DNA sequences were selected from a resource of tentatively identified clones from a porcine small intestine cDNA library. Forty ***PCR*** primer pairs were designed to amplify 101-309 base pairs of the 3' untranslated ***region*** of the genes. The ***PCR*** conditions were optimized by altering both form-amide and ***magnesium*** ***concns*** . on samples of pig, mouse, and hamster DNA. Twenty primer pairs that, under stringent conditions, were pig-specific and amplified the expected fragments were chosen for ***regional*** assignment in a pig/rodent hybrid cell panel. Furthermore, 22 primer pairs were chosen to amplify DNA from the parental animals of the PiGMaP shared ref. families in order to detect possible polymorphisms. Primer pairs that generated polymorphisms were used for genetic mapping. A total of 22 porcine expressed sequence tags (ESTs) were cytogenetically or genetically mapped by approach. Twelve of the mapped ESTs could be added to the human-porcine comparative map.

L27 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2002 ACS

1995:702964 Document No. 123:189430 Quantification of ***Mn*** -SOD mRNAs by using a competitive reverse-transcription ***polymerase*** ***chain*** ***reaction*** . Santiard-Baron, D.; Aral, B.; Ribiere, C.; Nordmann, R.; Sinet, P.-M.; Ceballos-Picot, I. (Departement de Recherches Biomedicales sur l'Alcoolisme, Universite R. Descartes, Paris, 75270, Fr.). Redox Report, 1(3), 185-9 (English) 1995. CODEN: RDRPE4.

ISSN: 1351-0002.

AB The ***manganese*** superoxide dismutase plays an important role in the cellular response to oxidative stress and appears to be highly regulated by many factors. The study of this gene's expression is difficult to achieve due to multiple rat ***Mn*** -SOD transcripts. In this report, we describe the quantification of the rat ***Mn*** -SOD transcripts by competitive reverse transcription- ***polymerase*** ***chain*** ***reaction*** . The competitor RNA was transcribed from a synthetic gene generated by ***PCR*** . This gene was composed of the T7 polymerase promoter linked to a 102 base-pair-deleted rat ***Mn*** -SOD cDNA. Both the target RNA and the competitor RNA were reverse-transcribed and co-amplified with the same primers. All the rat ***Mn*** -SOD mRNAs were simultaneously quantified by amplification of a common ***region*** . The use of a fluorescent primer led to fluorescent ***PCR*** products detected and quantified by the use of an automated DNA sequencer which avoides the use of radioactivity. Small variations in ***Mn*** -SOD mRNA ***concn*** . (30%) were detd. This method has been applied to study the expression of ***Mn*** -SOD mRNA in rat liver after chronic ethanol feeding. Expression of ***Mn*** -SOD transcripts was not modified and did not account for the increased ***Mn*** -SOD activity.

L27 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2002 ACS

1995:218791 Document No. 122:3291 Tumor multiplicity, DNA adducts and K-ras mutation pattern of 5-methylchrysene in strain A/J mouse lung. You, Liang; Wang, Dian; Galati, Anthony J.; Ross, Jeffrey A.; Mass, Marc J.; Nelson, Garret B.; Wilson, Katrina H.; Amin, Shantu; Stoner, Jason C.; et al. (Department Pathology, Medical College Ohio, Toledo, OH, 43699, USA). Carcinogenesis, 15(11), 2613-18 (English) 1994. CODEN: CRNGDP. ISSN: 0143-3334. Publisher: Oxford University Press.

AB This study was undertaken to evaluate the carcinogenic potential of 5-methylchrysene (5-MeC) in strain A/J mouse lung and to correlate the 5-MeC-DNA adduct profile in lung tissue with the mutation spectrum in the K-ras gene of lung tumors. Strain A/J mice received a single i.p. injection of 5-MeC at doses of 10, 50, 100 and 200 ***mg*** /kg and after 24, 48 and 72 h their lungs were collected for DNA adduct anal. Eight months later, lungs from the remaining mice were harvested and the lung tumors counted and collected for subsequent mutational anal. of the K-ras gene. 5-MeC was found to be a potent lung carcinogen in strain A/J mice, inducing more than 100 tumors/mouse at a ***concn*** . of 200 ***mg*** /kg. Six 5-MeC-DNA adducts were obsd.; one adduct comigrated with the std. N2-deoxyguanosine adduct of 5-MeC-diol-epoxide I [1R,2S,3S-trihydroxy-4R-(N2-deoxyguanosyl-3'-phosphate)-1,2,3,4-tetrahydro-5-methylchrysene], derived from the bay- ***region*** diol-epoxide of 5-MeC. DNAs isolated form 5-MeC-induced lung tumors were evaluated fro activating mutations in the K-ras gene by ***polymerase*** ***chain*** ***reaction*** -single strand conformation polymorphism and direct DNA sequencing anal. Mutations were detected in 44 of 49 (90%) 5-MeC-induced tumors and the mutations were GGT.fwdarw.TGT (50%), GGT.fwdarw.GTT (23%) and GGT.fwdarw.CGT (27%) in codon 12 of the gene. These results suggest that the N2-deoxyguanosine adduct of 5-MeC-diol-epoxide I may be one of the promutagenic adducts of 5-MeC in strain A/J mouse lung.

L27 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2002 ACS

1992:463969 Document No. 117:63969 Cloning of the 5' flanking ***region*** of the murine laminin B1 gene by genomic ***PCR*** . Okano, Ryusuke; Mita, Takashi; Matsui, Takashi (Sch. Med., Univ. Occup. and Environ. Health, Kitakyushu, 807, Japan). J. UOEH, 14(1), 23-31 (English) 1992. CODEN: JOUOD4. ISSN: 0387-821X.

AB Induction of the murine laminin B1 gene in F9 cells occurs 24-48 h after the retinoic acid (RA) addn. To study the mechanism of the late induction of the laminin B1 gene, the promoter was cloned and sequenced. The promoter ***region*** of the laminin B1 was isolated utilizing the genomic ***PCR*** technique. MgCl2, formamide concn., and annealing temp. were optimized for ***PCR*** . The result showed that MgCl2 concn. profoundly affects the efficiency in amplifying the specific DNA. The annealing temp. (51.degree.-63.degree.) did not significantly affect the yield. Under the optimal conditions, about 50 ng of the specific DNA was obtained from 1 .mu.g of total genomic DNA after 20 cycles of amplification, indicating that approx. 2 .times. 105 fold specific

amplification, indicating that approx. 2 .times. 105 fold specific amplification had occurred. Southern blot anal. and sequence data proved that the amplified DNA fragment contained the promoter ***region*** of the laminin B1 gene.

L27 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2002 ACS
1991:402460 Document No. 115:2460 The use of ***PCR*** to probe calcium channel diversity. Perez-Reyes, Edward; Wei, Xiangyang; Gudermann, Thomas; Birnbaumer, Lutz (Dep. Mol. Physiol., Baylor Coll. Med., Houston, TX, 77030, USA). J. Recept. Res., 11(1-4), 553-76 (English) 1991. CODEN: JRERDM. ISSN: 0197-5110.
AB Voltage-dependent calcium channels are a diverse set of proteins that can be classified into at least 3 classes based on their electrophysiol. and pharmacol. behavior. This report describes the development of a ***polymerase*** ***chain*** ***reaction*** (***PCR*** , Cetus) to probe for the expression of dihydropyridine-sensitive L-type calcium channel isoforms. The ***PCR*** reaction was optimized in terms of the following: methods for producing the template cDNA, ***concn*** . of primers, and ***magnesium*** ***concn*** . The factors involved in the design of oligonucleotides for ***PCR*** primers are discussed. These studies led to the following conclusions: 1) that primers should be less than 30 base pairs in length, 2) that the addn. of extraneous polylinker sequences on the 5' end of the primer has no effect, 3) that the primer should not be located in ***regions*** where secondary structure may exist, and 4) that non-degenerate primers can be used to amplify homologous gene family members. Methods are presented for subcloning ***PCR*** fragments, which allow the product of a single reaction to be subcloned and sequenced. The use of all these techniques is illustrated with RNA from mouse ovary, which expresses the cardiac isoform of the dihydropyridien-sensitive L-type calcium channel and a novel sequence postulated to be an isoform of L-type calcium channels.

L27 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2002 ACS
1990:585823 Document No. 113:185823 G to A substitution in the promoter ***region*** of the apolipoprotein AI gene is associated with elevated serum apolipoprotein AI and high density lipoprotein cholesterol concentrations. Jeenah, Mohammed; Kessling, Anna; Miller, Norman; Humphries, Steve (Charing Cross Sunley Res. Cent., London, W6 8LW, UK). Mol. Biol. Med., 7(3), 233-41 (English) 1990. CODEN: MBIMDG. ISSN: 0735-1313.
AB The sequence was detd. of 250 bases 5' of the transcriptional start site of the apolipoprotein AI gene in a human individual with high serum concns. of apo AI. One of the alleles contained a G to A substitution at position -75, between the CACAT sequence and the TAAATA box, creating a tandem repeat, CAGGGC-CA*GGGC. The substitution destroys an MspI cutting site, allowing the ***polymerase*** ***chain*** ***reaction*** and MspI digestion to identify the presence of the A or G base. The frequency of the A substitution in 96 healthy men from Bristol was 0.11, and this was increased to 0.25 in men with serum apo AI ***concns*** . >180 ***mg*** /dL. Men with the A allele had significantly higher serum concns. of apo AI, high-d. lipoprotein (HDL) cholesterol and HDL2 than those with the G allele. In this sample, variation assocd. with the G to A substitution accounted for 6% and 4.6% of the total variance in apo AI and HDL cholesterol concns., resp. Although there is as yet no functional proof, it is possible that the A substitution may be having a direct pos. effect on the rate of apo AI gene transcription and thus be assocd. with increased apo AI and HDL cholesterol concns. because of increased prodn. of apo AI protein from the liver or intestine.

```
=> S LIPASE
         37782 LIPASE
          6839 LIPASES
L30      39002 LIPASE
                (LIPASE OR LIPASES)

=> S L7 AND L30
L31         81 L7 AND L30

=> S L31 NOT (L22,L27)
L32         81 L31 NOT ((L22 OR L27))
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=> D 45-81 CBIB ABS

L32 ANSWER 45 OF 81 CAPLUS COPYRIGHT 2002 ACS
1997:757114 Document No. 128:58264 Novel DNA sequences provided by ***PCR*** amplification of hybrid genes. Dalboge, Henrik; Diderichsen, Borge; Sandal, Thomas; Kauppinen, Sakari (Novo Nordisk A/S, Den.; Dalboge, Henrik; Diderichsen, Borge; Sandal, Thomas; Kauppinen, Sakari). PCT Int. Appl. WO 9743409 A2 19971120, 71 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-DK216 19970512. PRIORITY: DK 1996-562 19960510.
AB The present invention relates to a method of providing novel DNA sequences encoding a polypeptide with an activity of interest, comprising the following steps: (1) ***PCR*** amplification of said DNA with ***PCR*** primers with homol. to (a) known gene(s) encoding a polypeptide with an activity of interest, (2) linking the obtained ***PCR*** product to a 5' structural gene sequence and a 3' structural gene sequence, (3) expressing said resulting hybrid DNA sequence, (4) screening for hybrid DNA sequences encoding a polypeptide with said activity of interest or related activity, (5) isolating the hybrid DNA sequence identified in step 4. Further, the invention also relates novel DNA sequences provided according to the method of the invention and polypeptides with an activity of interest encoded by said novel DNA sequences of the invention. The DNA sequences provided are full-length hybrid structural gene sequences encoding complete polypeptides with an activity of interest made up of one unknown sequence and one or two known sequences. Thus, conserved ***regions*** in known bacterial xylanase or cellulase sequences were identified by alignment and used to design ***PCR*** primers, and hybrid genes isolated by SOE- ***PCR*** (splicing by overlap extension- ***polymerase*** ***chain*** ***reaction***) from soil samples, cow rumen bacteria, and identified bacterial species.

L32 ANSWER 46 OF 81 CAPLUS COPYRIGHT 2002 ACS
1997:513690 Document No. 127:202065 ***Lipases*** of Absidia for use in laundry detergents and the genes encoding the enzymes. Berka, Randy M.; Boominathan, Karuppan C.; Sandal, Thomas (Novo Nordisk Biotech, Inc., USA; Novo Nordisk A/S). PCT Int. Appl. WO 9727276 A2 19970731, 61 pp. DESIGNATED STATES: W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US598 19970121. PRIORITY: US 1996-13616 19960124.
AB Genes encoding ***lipases*** of Absidia that are potentially of use in laundry detergents are cloned and characterized. The cloned genes can be used to manuf. the enzyme by expression in a host amenable to large-scale culture. ***Lipase*** genes of five Absidia species were cloned using ***PCR*** primers derived from conserved ***regions*** of other fungal ***lipases*** . Successful expression of the genes from the TAKA promoter in the prior art expression vector pBANE6 in Aspergillus oryzae as host is demonstrated.

L32 ANSWER 47 OF 81 CAPLUS COPYRIGHT 2002 ACS
1997:463986 Document No. 127:104909 Development of a direct DNA sequencing method for detecting heterozygous mutations of the human lipoprotein ***lipase*** gene. Mori, Atsuo; Takagi, Atsuko; Ikeda, Yasuyuki; Yamamoto, Akira (Department of Etiology and Pathophysiology, National Cardiovascular Center Research Institute, Osaka, 565, Japan). Clinical Biochemistry, 30(4), 315-324 (English) 1997. CODEN: CLBIAS. ISSN: 0009-9120. Publisher: Elsevier.
AB The purpose of this study was to develop an improved method of direct DNA sequencing, which makes it possible to identify heterozygous mutations of the lipoprotein ***lipase*** (L-PL) gene to understand the underlying genetic disorder of type IV hyperlipoproteinemia. The direct sequencing method was improved by devising primers for amplifying the LPL gene and for sequencing DNA amplified by the ***polymerase*** ***chain***

reaction (***PCR***), since the reported base sequences of the introns flanking exons of the LPL gene were limited to 40 bases. Improvement was achieved by attaching nine addnl. bases to both the ***PCR*** amplification primer and sequencing primer, and by optimizing the Tm value of the sequencing primers by adjusting the sequence of the nine extra bases. Use of the sequencing primers having suitable Tm values (48.degree. .apprx. 58.degree.) made it possible to reduce nonspecific bands on the sequence ladder pattern and to identify heterozygous mutation sites in LPL gene exons 5 and 6 as model cases. Our improved direct sequencing method is useful for identifying heterozygous mutation sites in human LPL gene exons and splicing consensus ***regions*** .

L32 ANSWER 48 OF 81 CAPLUS COPYRIGHT 2002 ACS

1997:117047 Document No. 126:182044 The sequence of a nearly unclonable 22.8 kb segment on the left arm of chromosome VII from Saccharomyces cerevisiae reveals ARO2, RPL9A, TIP1, MRF1 genes and six new open reading frames. Voet, Marleen; Defoor, Els; Verhasselt, Peter; Riles, Linda; Robben, Johan; Volckaert, Guido (Lab. Gene Tech., Katholieke Universiteit Leuven, Louvain, B-3001, Belg.). Yeast, 13(2), 177-182 (English) 1997. CODEN: YESTE3. ISSN: 0749-503X. Publisher: Wiley.

AB The nucleotide sequence of 22,803 bp on the left arm of chromosome VII was detd. by ***PCR*** -based approaches to compensate for the unstable character of cosmid clones from this ***region*** of the chromosome. The coding d. of the sequence is particularly high (>83%). Twelve open reading frames (ORFs) longer than 300 bp were found, 2 of which (at the left side) have been described previously after sequencing of an overlapping cosmid. Four other ORFs correspond to published sequences of the known genes ARO2, RPL9A, TIP1 and MRF1. ARO2 codes for chorismate synthetase, RPL9A for protein L9 of the large ribosomal subunit, and MRF1 for a mitochondrial translation release factor. The TIP1 product interacts with Sec20p and is thus involved in transport from endoplasmic reticulum to Golgi. Five of the remaining ORFs have not been identified previously, while the sixth (YGL142c) has been partially sequenced as it lies 5' upstream of MRF1. These six ORFs are relatively large (between 933 and 3657 nucleotides). YGL146c, YGL142c, YGL140c, and YGL139w have no significant homol. to any protein sequence presently available in the public databases, but show 2, 9, 9, and 8 putative transmembrane spans, resp. YGL144c has a serine active site signature of ***lipases*** . YGL141w has limited homol. to several human proteins, one of which mediates complex formation between papillomavirus E6 oncoprotein and tumor suppressor protein p53. The sequence reported in this paper has been deposited in the EMBL DNA data library under Accession No. X99960.

L32 ANSWER 49 OF 81 CAPLUS COPYRIGHT 2002 ACS

1996:718350 Document No. 126:3771 Mol. screening and ***PCR*** cloning of novel endoglucanases from fungi for use as detergents, textile treatment, and paper pulp processing. Schuelein, Martin; Andersen, Lene Nonboe; Lassen, Soeren Flensted; Kauppinen, Markus Sakari; Lange, Lene; Nielsen, Ruby Ilum; Ihara, Michiko; Takagi, Shinobu (Novo Nordisk A/s, Den.). PCT Int. Appl. WO 9629397 A1 19960926, 406 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-DK105 19960318. PRIORITY: DK 1995-272 19950317; DK 1995-885 19950808; DK 1995-886 19950808; DK 1995-887 19950808; DK 1995-888 19950808; DK 1996-137 19960212.

AB Cellulolytic enzymes (endoglucanases, cellulases) were isolated from such fungi as Myceliophthora thermophila, Acremonium, Thielavia terrestris, Macrophomina phaseolina, Crinipellis scabella, Volutella colletotrichoides, and Sordaria fimicola. The cDNAs for the enzymes were isolated characterized by mol. screening and ***PCR*** cloning using degenerate, deoxyinosine-contg. oligonucleotide primers corresponding to 4 highly conserved amino acid ***regions*** found in known amino acid sequences, and DNA constructs contg. the cDNAs were used to express the enzymes in transformed Aspergillus oryzae or A. niger. The enzyme prepns. consist essentially of an enzyme having cellulolytic activity and comprise a first amino acid sequence of 14 residues having the sequence Thr-Arg-X3-X4-Asp-Cys-Cys-X8-X9-X10-Cys-X12-Trp-X14, in which X3 and X4 independently is Trp, Tyr or Phe; X8 is Arg, Lys or His; each of X9, X10,

X12 and X13 is any of the 20 naturally occurring amino acid residues; and a second amino acid sequence of 5 residues having the sequence Trp-Cys-Cys-XX4-Cys in which XX4 is any of the 20 naturally occurring amino acid residues with the proviso that, in the first amino acid sequence, (i) when X12 is Ser, then X14 is not Ser, and (ii) when X12 is Gly, then X14 is not Ala. Gene fusions were also constructed between endoglucanases from Myceliophthora thermophila, Macrophomina phaseolina, and Crinipellis scabella and the linker/cellulose-binding C-terminal ***region*** of the endoglucanase from Humicola insolens. The enzymes perform excellently in detergent, laundering, textile, and papermaking pulp applications. ***PCR*** -facilitated detection of cellulolytic enzymes and their cDNA sequences are also described from 46 filamentous and monocentric fungi representing 32 genera.

L32 ANSWER 50 OF 81 CAPLUS COPYRIGHT 2002 ACS

1996:643666 Document No. 126:99915 The improvement of a direct DNA sequencing method by devising the primer design. Detection of lipoprotein ***lipase*** gene aberrations. Mori, Atsuo; Takagi, Atsuko; Ikeda, Yasuyuki; Yamamoto, Akira (Dep. Etiol. Pathophysiol., Natl. Cardiovasc. Cent. Res. Inst., Suita, 565, Japan). Rinsho Byori, 44(10), 983-990 (Japanese) 1996. CODEN: RBYOAI. ISSN: 0047-1860. Publisher: Rinsho Byori Kankokai.

AB We developed an improved method of direct DNA sequencing which makes it possible to detect heterozygous mutations in an exon and the splicing consensus ***regions*** (acceptor and donor) in introns even in the cases where there is limited information available regarding the base sequences of the introns flanking the exon. Human lipoprotein ***lipase*** (LPL) gene was utilized to develop this method, since the reported intron base sequences of the LPL gene are limited to 40 bases. We constructed ***PCR*** primers with extra 9 bases for the LPL gene amplification and 20-mers from 5' end of the ***PCR*** primers were used as sequencing primers in order to obtain the necessary distance from the 3' end of the sequencing primer to 5' end of the splicing acceptor consensus sequence for the accurate detn. of the sequence of the consensus ***region*** . Furthermore, the attachment of the 9 bases made it possible to optimize the Tm value of the sequencing primers by adjusting their (G + C)/(A + T) ratio. The direct sequencing method using the sequencing primer with an appropriate Tm value (i.e., 48.degree.C .apprx. 58.degree.C in this study) was effective in reducing nonspecific bands on the sequence ladder pattern, and allowed the detn. of the base sequences of both the sense and antisense strands of the splicing consensus sequences and exon. As a result, this improved direct sequencing method was capable of detecting heterozygous as well as homozygous LPL gene mutations.

L32 ANSWER 51 OF 81 CAPLUS COPYRIGHT 2002 ACS

1996:386040 Document No. 125:50739 Method, reagent and kit for evaluating susceptibility to premature atherosclerosis and its treatment using human lipoprotein ***lipase*** gene therapy. Hayden, Michael R.; Ma, Yuanhong; Lewis, Suzanne; Liu, Guoquing (University of British Columbia, Can.). PCT Int. Appl. WO 9611276 A1 19960418, 37 pp. DESIGNATED STATES: W: CA, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US13620 19951011. PRIORITY: US 1994-320604 19941011.

AB A single point mutation in the human lipoprotein ***lipase*** gene which results in an A .fwdarw. G nucleotide change at codon 291 (nucleotide 1127) of the lipoprotein ***lipase*** gene, and a substitution of serine for the normal asparagine in the lipoprotein ***lipase*** gene product is seen with increased frequency in patients with coronary artery disease, and is assocd. with an increased susceptibility to coronary artery disease, including in particular premature atherosclerosis. This is expressed as a diminished catalytic activity of lipoprotein ***lipase*** , lower HDL-cholesterol levels, and higher triglyceride levels. Thus, susceptibility of a human individual to premature atherosclerosis can be evaluated by: (a) obtaining a sample of DNA from the individual; and (b) evaluating the sample of DNA for the presence of nucleotides encoding a serine residue as amino acid 291 of the lipoprotein ***lipase*** gene product. Thus, the mismatched primer 5'-ctgcttcttttggctctgactgta-3' can be used in ***PCR*** or strand displacement amplification of the mutant gene ***region*** . Patients found to be suffering from or likely to suffer

from premature atherosclerosis and other forms of coronary artery disease as a result of a lipoprotein ***lipase*** deficiency can be treated using gene therapy. Thus, viral vectors were constructed for gene therapy using an E1 deletion mutant adenovirus, polylysine, and a plasmid contg. human lipoprotein ***lipase*** cDNA under control of the cytomegalovirus promoter ***region*** . Vectors for introducing human lipoprotein ***lipase*** cDNA into mammalian cells were made using the murine leukemia retroviral backbones M3neo, M5neo, and JZen1 which contain long terminal repeat regulatory sequences for the myeloproliferative sarcoma virus. A 1.56-kb DraI-EcoRI fragment encompassing the entire ***lipase*** amino acid coding ***region*** was subcloned into a unique BamHI site located 3' or 5' to the neomycin phosphotransferase gene. Gene transfer efficiency was up to 57% with an increase in lipoprotein ***lipase*** bioactivity up to 14-fold and an increases in enzyme dimer up to 54-fold.

L32 ANSWER 52 OF 81 CAPLUS COPYRIGHT 2002 ACS

1996:381602 Document No. 125:82690 Endothelial cells synthesize and process apolipoprotein B. Sivaram, Pillarisetti; Vanni-Reyes, Teresa; Goldberg, Ira J. (Dep. Med., Columbia Univ., New York, NY, 10032, USA). Journal of Biological Chemistry, 271(25), 15261-15266 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB We reported previously that a 116-kDa lipoprotein ***lipase*** (LPL)-binding protein from endothelial cells has sequence homol. to the amino-terminal ***region*** of apolipoprotein (apo) B. We now tested whether endothelial cells synthesize apoB mRNA and protein. Primers were designed to the human apoB cDNA sequence and reverse transcription ***polymerase*** ***chain*** ***reaction*** was performed using total RNA isolated from bovine and human endothelial cells. With primers to the 5' ***region*** of the apoB mRNA (amino-terminal ***region*** of apoB protein) expected size ***PCR*** products were generated from both bovine and human endothelial cells as well as from mouse liver RNA, which was used as a control. Primers designed to the 3' ***region*** of apoB mRNA generated ***PCR*** products from human endothelial cells and HepG2 cells but not from bovine or mouse cells. These data suggest that endothelial cells contain full-length apoB mRNA and that the 5' or the amino-terminal ***region*** of apoB is highly conserved from mouse to human. This was confirmed by direct sequencing of the mouse and bovine ***PCR*** products. To test whether apoB protein was produced bovine endothelial cell proteins were metabolically labeled with [35S]methionine/cysteine or [3H]leucine and immunopptd. with anti-human apoB antibodies. Using exts. from cells labeled for 1 h, monoclonal antibody 47, directed to the low d. lipoprotein receptor binding ***region*** of apoB, pptd. a protein of approx. mol. mass 550,000, the size of full-length apoB. Immunopptn. of the 550-kDa protein was abolished in the presence of added unlabeled low d. lipoprotein. From cells labeled for 16 h, a 116-kDa protein was immunopptd. by polyclonal anti-apoB antibodies. This protein was partly released from cells by heparin treatment. Pulse-chase anal. showed that the 116-kDa fragment appeared at the same time as the full-length apoB began disappearing. The immunopptd. 116-kDa fragment also bound labeled LPL on ligand blot, further suggesting that it is an amino-terminal fragment of apoB. Incubation of endothelial cells with oleic acid (0.25 and 0.5 mM) did not significantly alter the prodn. of either the full-length apoB or the 116-kDa fragment. These data show that endothelial cells synthesize apoB. The full-length apoB appears to be cleaved to form a 116-kDa fragment that can function as a LPL-binding protein.

L32 ANSWER 53 OF 81 CAPLUS COPYRIGHT 2002 ACS

1996:339500 Document No. 125:54151 Lipoprotein ***lipase*** in highly vascularized structures of the eye. Casaroli-Marano, Ricardo P.; Peinado-Onsurbe, Julia; Reina, Manuel; Staels, Bart; Auwerx, Johan; Vilaro, Senen (Dep. of Cell Biology, Univ. of Barcelona, Barcelona, 08028, Spain). Journal of Lipid Research, 37(5), 1037-1044 (English) 1996. CODEN: JLPRAW. ISSN: 0022-2275. Publisher: Lipid Research, Inc..

AB Ocular tissues are highly dependent on lipid turnover and metab., which requires an uptake mechanism for fatty acids from lipoproteins. The authors studied the activity and expression of lipoprotein ***lipase*** (LPL), which catalyzes the hydrolysis of plasma triglycerides in different ocular ***regions*** . Human and bovine eyes are dissected and various

specialized anatomical areas were assayed for LPL activity, mRNA, and immunoreactivity. Variable levels of LPL activity were detected in all structures in human and bovine eyes. LPL activity was much higher in vascularized structures, such as ciliary body, iris, and retina, than in avascular eye structures, such as vitreous body, lens, and cornea. In both human and bovine eyes, ciliary body contained the highest LPL lipolytic activity. LPL mRNA was detected by reverse transcription followed by ***polymerase*** ***chain*** ***reaction*** (RT-***PCR***) in all ***regions*** of human eyes. By RT- ***PCR*** anal. it was shown that bovine eyes contained high levels o LPL mRNA in ciliary body and iris, lower levels were found in retina, optic nerve, and lens, whereas no LPL mRNA could be found in bovine cornea. RT- ***PCR*** data, obtained in bovine eyes, agree with th results obtained by Northern blot expts., confirming the high levels of LPL mRNA in iris and ciliary body. Immunofluorescence expts. performed on human eye samples indicated that the LPL protein is mostly distributed on the choroides, the choriocapillaris, and on the vessels of ciliary body, iris, optic nerve, and retina. The present study demonstrates that active LPL protein is synthesized, secreted, and located among microvessels in several specialized ***regions*** of the eye, and suggests that LPL could be involved in the uptake of fatty acids by the ocular tissues.

L32 ANSWER 54 OF 81 CAPLUS COPYRIGHT 2002 ACS

1995:791285 Document No. 123:331510 Baboon lipoprotein ***lipase*** : cDNA sequence and variable tissue-specific expression of two transcripts. Cole, Shelley A.; Hixson, James E. (Southwest Foundation for Biomedical Research, San Antonio, TX, 78228-0147, USA). Gene, 161(2), 265-9 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB We have isolated two lipoprotein ***lipase*** (LPL)-encoding cDNA (LPL) clones from a baboon cardiac cDNA library, one of which spans a ***region*** from nucleotide (nt) 705 of the coding sequence to the poly(A) tail (2.8kb). We used reverse transcription followed by ***PCR*** (RT- ***PCR***), and anchor-ligated rapid amplification of cDNA ends (RACE) to amplify the remaining 5' ***region*** of the LPL transcript. Sequence comparisons reveal that the baboon nt sequence is 95% identical to the human cDNA sequence (ranging from 97.5 to 92.7% in the coding and noncoding ***regions*** , resp.). Less than 2% of nt substitutions cause changes between baboon and human amino acid (aa) sequences. The aa in the catalytic triad residues, the heparin-binding site in exon 6, as well as aa in positions where missense mutations cause LPL deficiency, are identical in baboons and humans. Characterization of the tissue-specific expression of LPL using Northern blots of total RNA showed that spinal cord expressed the most LPL transcripts of all baboon tissues examd. Like humans, baboons have two transcript sizes of approx. 3.6 and 3.4 kb in most tissues that express LPL, and sequencing of the 3' untranslated ***region*** (UTR) shows this is due to two polyadenylation sites. In contrast, only the larger 3.6-kb transcript is detected in RNA isolated from central nervous system (CNS) tissues. We used RT- ***PCR*** to show that the polyadenylation signal that produces the 3.4-kb message is present in CNS LPL transcripts, but is not utilized.

L32 ANSWER 55 OF 81 CAPLUS COPYRIGHT 2002 ACS

1995:381421 Document No. 122:180021 A 5' splice- ***region*** mutation and a dinucleotide deletion in the lysosomal acid ***lipase*** gene in two patients with cholesteryl ester storage disease. Ameis, Detlev; Brockmann, Gerald; Knoblich, Rupert; Merkel, Martin; Ostlund, Richard E., Jr.; Yang, Joseph W.; Coates, Paul M.; Cortner, Jean A.; Feinman, S. Victor; Greten, Heiner (Department of Medicine, University Hospital Eppendorf, Hamburg, 20246, Germany). Journal of Lipid Research, 36(2), 241-50 (English) 1995. CODEN: JLPRAW. ISSN: 0022-2275. Publisher: Lipid Research, Inc..

AB Cholesteryl ester storage disease (CESD) results from inherited deficiencies of the lysosomal hydrolase, acid ***lipase*** (LAL; E.C. 3.1.1.13). To establish the mol. defects in LAL deficiency, two unrelated probands with severely reduced LAL activity were examd. DNA amplification by reverse-transcription ***polymerase*** ***chain*** ***reaction*** and subsequent sequence anal. of LAL cDNA identified two mutant alleles. Patient 1, presenting with hepatosplenomegaly, mildly elevated liver function tests, and hyperlipidemia, was homozygous for a deletion of nucleotides 823 and 894 of the LAL cDNA. This 72-bp deletion

maintained the reading frame and resulted in a loss of 24 amino acids from the LAL protein. Anal. of genomic DNA revealed that the 72 bp corresponded to an exon of the LAL gene. A single G to A point mutation at the last exon position was obsd. in the genomic DNA of patient 1, indicating a splicing defect with consecutive exon skipping underlying the 72-bp deletion. Patient 2 was a component heterozygote for the 72-bp deletion and a dinucleotide deletion at positions 967 and 968. This deletion resulted in a shifted reading frame carboxy-terminal of codon 296, and 43 random amino acids followed the frame shift. A premature stop at codon 339 truncated the mutant LAL protein by 34 amino acids. Allele-specific hybridization confirmed that patient 1 was homogeneous for the 72-bp deletion mutation, and that patient 2 was a compd. heterozygote for the 72-bp deletion and the 2-bp deletion.

L32 ANSWER 56 OF 81 CAPLUS COPYRIGHT 2002 ACS
1995:266922 Document No. 122:152928 Yeast artificial chromosome and radiation hybrid map of loci in chromosome band 8p22, a common ***region*** of allelic loss in multiple human cancers. Bookstein, Robert; Levy, Alina; MacGrogan, Donal; Lewis, Tracey B.; Weissenbach, Jean; O'Connell, Peter; Leach, Robin J. (Canji, Inc., San Diego, CA, 92121, USA). Genomics, 24(2), 317-23 (English) 1994. CODEN: GNMCEP. ISSN: 0888-7543. Publisher: Academic.
AB Polymorphic alleles at loci such as LPL (lipoprotein ***lipase***) and MSR (macrophage scavenger receptor) in chromosome band 8p22 are frequently lost during the genesis of several types of human cancer, including colorectal, non-small cell lung, hepatocellular, and prostatic carcinomas. A phys. map of 31 published or novel probes and sequence-tagged sites in this genetic ***region*** was constructed using a radiation hybrid panel and the CEPH (Center d'Etude du Polymorphisme Humain) yeast artificial chromosome (YAC) library. Thirty-six overlapping YACs defined a phys. order for the following polymorphic markers: tel-D8S26 - D8S511 - DS549 - MSR - D8S254 - D8S233 - D8S261 - D8S21 - LPL - D8S258-cen. These maps unify small consensus ***regions*** of allelic loss on chromosome 8p defined by restriction fragment length polymorphisms with more informative ***PCR*** -based polymorphisms and widely available YAC mapping resources.

L32 ANSWER 57 OF 81 CAPLUS COPYRIGHT 2002 ACS
1995:191542 Document No. 122:257425 ***Regional*** localization of human chromosome 15 loci. Richard, Isabelle; Broux, Odile; Chiannilkulchai, Nuchanard; Fougerousse, Francoise; Allamand, Valerie; Bourg, Nathalie; Brenguier, Lydie; Devaud, Catherine; Pasturaud, Patricia; et al. (Localization Department, Genethon, Evry, 91000, Fr.). Genomics, 23(3), 619-27 (English) 1994. CODEN: GNMCEP. ISSN: 0888-7543. Publisher: Academic.
AB One hundred forty-nine chromosome 15 loci were mapped by ***PCR*** with respect to chromosome breakpoints in 3 somatic cell hybrids retaining total or part of chromosome 15 and to a 10-Mb YAC contig. This chromosome was subdivided into 5 ***regions*** , yielding an av. resoln. of more than 1 sequence tagged site per megabase. The mapped loci included 18 genes, 60 cDNA-derived sequence tagged sites, and 69 microsatellites. In addn., the amt. of chromosome 15 retained in line A15.1 has been defined. This work represents the first attempt at an integration of the human phys., expression, and genetic maps of chromosome 15.

L32 ANSWER 58 OF 81 CAPLUS COPYRIGHT 2002 ACS
1995:141060 Document No. 122:73608 The cDNA sequence encoding bovine pregastric esterase. Timmermans, Miet Y. J.; Teuchy, Henri; Kupers, Luc P. M. (Department of Biochemistry, Limburgs Universitair Centrum, B-3590, Diepenbeek, Belg.). Gene, 147(2), 259-62 (English) 1994. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.
AB The ***polymerase*** ***chain*** ***reaction*** (***PCR***) was used to amplify specific parts of the gene encoding calf pregastric esterase (PGE). Primers based on conserved ***regions*** in human gastric ***lipase*** (HGL) and rat lingual ***lipase*** (RLL) were used to screen a cDNA library prepd. from calf tongue tissue. This resulted in the cloning of the entire coding sequence for PGE, which exists as a mature 378-amino-acid (aa) polypeptide with a mol. mass of 42960 Da. The PGE, HGL and RLL genes all share a high degree of identity at both the nucleotide and amino-acid sequence levels. Except for the Gly-Xaa-Ser-Xaa-Gly sequence contg. the active site Ser, there is little

identity with non-preduodenal ***lipases*** .

L32 ANSWER 59 OF 81 CAPLUS COPYRIGHT 2002 ACS
1995:115470 Document No. 122:179518 Determination of changes in specific gene expression by reverse transcription ***PCR*** using interspecies mRNA as internal standards. Levesque, G.; Lamarche, B.; Murthy, M. R. V.; Julien, P.; Despres, J. P.; Deshaies, Y. (Laval Univ., QC, Can.). BioTechniques, 17(4), 738-41 (English) 1994. CODEN: BTNQDO. ISSN: 0736-6205.
AB A method is described for the detn. of changes in gene expression by reverse transcription of the target mRNA followed by ***PCR*** amplification of the resulting cDNA (RT- ***PCR***), using the lipoprotein ***lipase*** gene as the model system. Known proportions of human and rat adipose tissue homogenates are mixed and are processed together throughout the RT- ***PCR*** procedure so that the rat tissue serves as an internal std. for the measurement of human adipose tissue lipoprotein ***lipase*** (LPL) in all steps including RNA extn., reverse transcription and ***PCR*** amplification. Taking advantage of the highly conserved sequence of the LPL gene across species, selected homologs ***regions*** of the human and rat genes are amplified using the same primer pair and resulting in the same lengths of amplified DNA fragments. The two amplified products are then sepd. from each other by making use of differences in the position of a restriction site in the two amplified, precise and reproducible and avoids construction of tailored nucleic acids for use as internal stds.

L32 ANSWER 60 OF 81 CAPLUS COPYRIGHT 2002 ACS
1995:30798 Document No. 122:98217 Chromosomal localization of human genes for the LDL receptor family member glycoprotein 330 (LRP2) and its associated protein RAP (LRPAP1). Korenberg, Julie R.; Argraves, Kelley M.; Chen, Xiao-Ning; Tran, Huan; Strickland, Dudley K.; Argraves, W. Scott (Biochem. Dep., American Red Cross, Rockville, MD, 20855, USA). Genomics, 22(1), 88-93 (English) 1994. CODEN: GNMCEP. ISSN: 0888-7543.
AB Glycoprotein 330 (gp330) is a member of a family of receptors with structural similarities to the low-d. lipoprotein receptor. Gp330 is expressed by a no. of specialized epithelia, including renal proximal tubules, where it can mediate endocytosis of ligands such as complexes of urokinase and the serpin, plasminogen activator inhibitor-1. Gp330 has also been shown to bind in vitro to lipoprotein ***lipase*** and apolipoprotein E-enriched .beta.VLDL, suggesting a role for this receptor in lipoprotein metab. The 30-kDa protein, referred to as receptor-assocd. protein (RAP), binds to and copurifies with gp330 and antagonizes the ligand binding activity of gp330. In this paper, the authors report the use of homol.- ***PCR*** cloning to isolate cDNAs encoding human gp330. Using gp330 cDNA and previously isolated human RAP cDNA probes, the authors performed fluorescence in situ hybridization to map the human chromosomal location of the genes for these proteins. The gene for gp330 was mapped at a single site on the long arm of human chromosome 2 on the borer of bands 2q24-q31. The gene for RAP was mapped to the short arm of human chromosome 4 at position 4p16.3, which is in the ***region*** of the chromosomal deletion causing Wolf-Hirschhorn syndrome. The assignment of chromosomal map positions for gp330 and RAP genes will aid in the evaluation of their potential roles in human diseases such as Wolf-Hirschhorn syndrome and disorders of lipoprotein metab., such as atherosclerosis.

L32 ANSWER 61 OF 81 CAPLUS COPYRIGHT 2002 ACS
1994:648894 Document No. 121:248894 A novel missense mutation in the C-terminal domain of lipoprotein ***lipase*** (Glu410 .fwdarw. Val) leads to enzyme inactivation and familial chylomicronemia. Previato, Lorenzo; Guardamagna, Ornella; Dugi, Klaus A.; Ronan, Rosemary; Talley, Glenda D.; Santamarina-Fojo, Silvia; Brewer, H. Bryan, Jr. (Mol. Dis. Branch, Natl. Heart, Lung, Blood Inst., Bethesda, MD, 20892, USA). Journal of Lipid Research, 35(9), 1552-60 (English) 1994. CODEN: JLPRAW. ISSN: 0022-2275.
AB Lipoprotein ***lipase*** (LPL) is a complex enzyme consisting of multiple functional domains essential for the initial hydrolysis of triglycerides present in plasma lipoproteins. Previous studies have localized the catalytic domain of LPL, responsible for the hydrolytic function of the enzyme, to the N-terminus whereas the C-terminal end may play a role in lipid and heparin binding. To date, most described

missense mutations resulting in a nonfunctional LPL have been located in the N-terminal ***region*** of the enzyme. The authors describe the defect in the LPL gene of a patient with triglycerides ranging from normal to 12,000 mg/dL, low LPL mass, and no LPL activity in post-heparin plasma. Sequencing of patient ***PCR*** -amplified DNA identified two sep. mutations in the C-terminal domain of LPL: an A .fwdarw. T transversion at nucleotide 1484 resulting in a Glu410 .fwdarw. Val substitution and a C .fwdarw. G mutation at position 1595 that introduces a premature stop codon at position 447. Digestion with MaeIII and MnII established that the patient is a true homozygote for both mutations. In order to investigate the functional significance of these defects, mutants enzymes contg. either the Val410 or the Ter447 mutations as well as both Val410 and Ter447, were expressed in vitro. Compared to the wild-type enzyme, LPL447 demonstrated a moderate of specific activity using triolein (70% of normal) and tributyrin (74% of normal) substrates, while LPL410 had a significant (11% and 23% of normal) redn. of the normal ***lipase*** and esterase specific activities, resp. Mutant-LPL410/447 was virtually inactive using either triolein or tributyrin substrates establishing the functional significance of this combined defect. When analyzed by heparin-Sepharose affinity chromatog., a small fraction of LPL410, like the native LPL dimer, eluted at an NaCl concn. of 1.3M and had a normal specific activity. However, most of the LPL410 mass was detected in an inactive peak that, like the normal LPL-monomer, eluted at 0.8 M NaCl, indicating that the Glu410 .fwdarw. Val substitution may alter the stability of the LPL dimer.

L32 ANSWER 62 OF 81 CAPLUS COPYRIGHT 2002 ACS

1994:451366 Document No. 121:51366 Hepatic ***lipase*** gene is transcribed in rat adrenals into a truncated mRNA. Verhoeven, Adrie J. M.; Carling, Dave; Jansen, Hans (Dep. Biochem., Erasmus Univ., Rotterdam, Neth.). Journal of Lipid Research, 35(6), 966-75 (English) 1994. CODEN: JLPRAW. ISSN: 0022-2275.

AB Rat adrenals contain a ***lipase*** activity that is indistinguishable from hepatic ***lipase*** (HL) present in liver. Expression of HL mRNA in adrenals was studied using the method of reverse transcription-***polymerase*** ***chain*** ***reaction*** (RT- ***PCR***). A 596-bp fragment of HL cDNA spanning exons 5 to 8 was amplified when using total RNA from rat adrenals and liver, but not from heart or kidney. The abundance of HL mRNA was quantified by competitive RT- ***PCR*** using a std. RNA that was generated in vitro by transcription from a deleted HL cDNA construct. Adrenals contained 0.4 amol of HL mRNA per .mu.g of total RNA, compared to 16 amol in liver. In hypertrophic adrenals isolated from corticotrophin treated rats, the abundance also amounted to 0.4 amol of mRNA per .mu.g of total RNA. However, amplification of full-length cDNA from either control or hypertrophic adrenals was never obsd. Detailed anal. by ***PCR*** using different combinations of primers indicated that exons 3 to 9 including the 3'-untranslated ***region*** were expressed in adrenal RNA, but not the first two coding exons. The upstream part of the adrenal ***lipase*** mRNA was cloned after rapid amplification of cDNA ends (RACE). The resulting clones showed a unique 126-bp sequence 5' of the exon 2-exon 3 junction. This sequence contained multiple termination codons in all three reading frames but lacked a potential start codon. RT- ***PCR*** using an HL-specific primer and an oligonucleotide directed against this 5'-sequence showed that it is not only expressed in RNA from adrenals but also in liver. Pulse-labeling of freshly isolated adrenocortical cells with [35S]methionine followed by immunopptn. with anti-HL antibodies failed to show synthesis of mature HL, but indicated the synthesis of immunoreactive proteins in the 40-45 kDa range that remained mainly intracellular. Hence, the HL gene is transcribed in adrenals but results in an mRNA species with a unique 5'-end. Translation from an internal start site may produce an intracellular HL isoform that differs markedly from the liver-type ***lipase*** previously identified in adrenals.

L32 ANSWER 63 OF 81 CAPLUS COPYRIGHT 2002 ACS

1994:155332 Document No. 120:155332 The human carboxyl ester ***lipase*** -like (CELL) gene is ubiquitously expressed and contains a hypervariable ***region*** . Nilsson, Jeanette; Hellquist, Marika; Bjursell, Gunnar (Dep. Mol. Biol., Univ. Goeteborg, Goeteborg, S-413 90, Swed.). Genomics, 17(2), 416-22 (English) 1993. CODEN: GNMCEP. ISSN: 0888-7543.

AB The human carboxyl ester ***lipase*** -like (CELL) transcript was characterized. An anal. of the tissue distribution of the expression of the gene shows that it is expressed in low amts. in all tissues analyzed. This is in contrast to its closely related and functional gene, the carboxyl ester ***lipase*** (CEL) gene, which is expressed only in human lactating mammary gland and pancreas. The primary structure of the cDNA encoding the carboxyl ester ***lipase*** -like transcript was detd. The av. length of the cDNA is 1214 bases. This sequence includes several termination codons in all 3 reading frames. The longest open reading frame with the same start of translation as that of the CEL transcript could encode a 59-amino-acid-long peptide, presumably without any function. The CELL gene may have arisen as a result of a gene duplication of the CEL gene followed by deletions and point mutations. However, the mutations are unevenly distributed. In the first 3 exons no mutations are found compared to the corresponding exons of the CEL gene. On the other hand, in the next exon several point mutations and a 2-base insertion are found and are present in all individuals analyzed. A hypervariable ***region*** present in the last exon of the CELL gene is also characterized. Several allelic variants can be resolved by ***PCR*** amplification of this ***region*** followed by sequencing using an automated laser fluorescent sequencer. All alleles have a base substitution at position 906 and differ with respect to 3 different sequence boxes; they either have or lack one or several boxes. Seven different alleles were distinguished in a study of 9 alleles. This new hypervariable ***region*** should be applicable to pedigree and linkage analyses.

L32 ANSWER 64 OF 81 CAPLUS COPYRIGHT 2002 ACS
1994:51804 Document No. 120:51804 Recurrent pancreatitis and chylomicronemia in an extended Dutch kindred is caused by a Gly154.fwdarw.Ser substitution in lipoprotein ***lipase*** . Bruin, Taco; Tuzgol, Suat; Van Diermen, Denise E.; Hoogerbrugge-Van der Linden, Nicoline; Brunzell, John D.; Hayden, Michael R.; Kastelein, John J. P. (Acad. Med. Cent., Univ. Amsterdam, Amsterdam, Neth.). Journal of Lipid Research, 34(12), 2109-19 (English) 1993. CODEN: JLPRAW. ISSN: 0022-2275.

AB The authors report the mol. basis of familial chylomicronemia and recurrent pancreatitis in five members of a large Dutch family. All patients had normal plasma hepatic ***lipase*** and apoC-II levels, but absent lipoprotein ***lipase*** (LPL) catalytic activity and low LPL mass in postheparin plasma. The mutation in the LPL gene was characterized as a G715.fwdarw.A substitution in the last nucleotide of exon 4, resulting in a substitution of Ser for Gly154. ***PCR*** amplification of exons 4 + 5 from the patients' mRNA, followed by direct sequencing, revealed normal splicing of intron 4. The mutation creates a BfaI restriction site that allows rapid screening of family members for the mutation. Reprodn. of this mutation in LPL-cDNA by site-directed mutagenesis, followed by transient expression in COS-B cells, revealed prodn. of a catalytically inactive enzyme. The Gly154.fwdarw.Ser substitution appears in a conserved beta-sheet ***region*** , in close proximity to Asp156, which is part of the catalytic triad. Thus, changes to residues close to Asp156 can have profound effects on catalytic activity of LPL.

L32 ANSWER 65 OF 81 CAPLUS COPYRIGHT 2002 ACS
1993:510136 Document No. 119:110136 Cloning, nucleotide sequence and expression in Escherichia coli of a ***lipase*** gene from Bacillus subtilis 168. Dartois, Veronique; Baulard, Alain; Schanck, Karin; Colson, Charles (Unite Genet., Univ. Cathol. Louvain, Louvain-la-Neuve, Belg.). Biochimica et Biophysica Acta, 1131(3), 253-60 (English) 1992. CODEN: BBACAQ. ISSN: 0006-3002.

AB The gene coding for an extracellular ***lipase*** of B. subtilis 168 was cloned and found to be expressed in E. coli. Enzyme activity measurements showed no fatty acid chain length preference. A set of Tn5 insertions which inactivate the gene were localized and used to initiate its sequencing. The nucleotide sequence was detd. on two independent clones expressed in E. coli. In one of these clones, the sequence revealed a frameshift, due to the presence of an addnl. adenine in the N-terminal ***region*** , which caused the interruption of the open reading frame, probably allowing translation to initiate at a second ATG codon. The sequence of the wild-type lip gene from B. subtilis was confirmed on the chromosomal fragment amplified by ***polymerase***

chain ***reaction*** (***PCR***). When compared to other ***lipases*** sequenced to date, the enzyme described here lacks the conserved pentapeptide Gly-X-Ser-X-Gly supposed to be essential for catalysis. However, alignments of several microbial ***lipase*** sequences suggest that the pentapeptide Ala-X-Ser-X-Gly present in the ***lipase*** of B. subtilis may function as the catalytic site. Homologies were found in the N-terminal protein ***region*** with ***lipases*** from different Pseudomonas species. The predicted Mr and isoelec. point for the mature protein are 19,348 and 9.7 resp.

L32 ANSWER 66 OF 81 CAPLUS COPYRIGHT 2002 ACS
1993:249309 Document No. 118:249309 Cloning and expression of Penicillium gene for partial glycerides-specific ***lipase*** . Yamaguchi, Shotaro; Isobe, Kimyasu; Takeuchi, Kazuyuki (Amano Pharma Co., Ltd., Japan). Jpn. Kokai Tokkyo Koho JP 05041991 A2 19930223 Heisei, 12 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1991-280626 19910930. PRIORITY: JP 1990-269198 19901005.
AB The gene for partial glycerides (i.e., mono- and di-glycerides)-specific ***lipase*** (I) that does not react with triglycerides is cloned from the genome of Penicillium sp. and expressed in Saccharomyces and Aspergillus. From a genomic library of Penicillium sp., the gene for I was cloned using a DNA probe prepd. by ***PCR*** using primers derived from the protein sequence of I. The I gene was used to construct plasmids from YEp13 for expression in S. cerevisiae. The yield of I with the recombinant S. cerevisiae was 4.2 unit/mL. Also shown was the expression of I gene in A. oryzae by co-transfection with the niaD marker gene-contg. plasmid pSTA14.

L32 ANSWER 67 OF 81 CAPLUS COPYRIGHT 2002 ACS
1993:206117 Document No. 118:206117 Mismatch ***PCR*** : a rapid method to screen for the Pro207 .fwdarw. Leu mutation in the lipoprotein ***lipase*** (LPL) gene. Bijvoet, Saskia M.; Hayden, Michael R. (Dep. Med. Genet., Univ. British Columbia, Vancouver, BC, V6T 1ZA, Can.). Hum. Mol. Genet., 1(7), 541 (English) 1992. CODEN: HMGEE5. ISSN: 0964-6906.
AB LPL deficiency occurs with highest worldwide frequency in the province of Quebec, Canada, where the carrier rate is 1 in 40 in some ***regions*** . Here a new method which allows more rapid and efficient screening for the Pro207.fwdarw.Leu mutation in the LPL gene, the most frequently encountered mol. defect underlying LPL deficiency in the French Canadian population is reported.

L32 ANSWER 68 OF 81 CAPLUS COPYRIGHT 2002 ACS
1993:142540 Document No. 118:142540 Stabilization of enzymes by selective replacement of amino acids with proline. Svendsen, Allan; Von der Osten, Claus; Clausen, Ib Groth; Patkar, Shamkant Anant; Borch, Kim (Novo Nordisk A/S, Den.). PCT Int. Appl. WO 9219726 A1 19921112, 45 pp. DESIGNATED STATES: W: FI, JP, KR, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-DK142 19920501. PRIORITY: EP 1991-610035 19910501.
AB Enzymes are made more stable by methods that minimize the structural flexibility of the peptide backbone. This is achieved by substituting selected amino acids with proline in ***regions*** of the protein outside stable higher order structures such as .alpha.-helices and .beta.-sheets. The criteria for selecting amino acids to be substituted also include dihedral angles .psi. and .phi. in the ranges -90.degree.<.phi.<-40.degree. and -180.degree.<.psi.<-150.degree., -80<.psi.<10, 100.degree.<.psi.<180. The invention is intended for use in the prepn. of stable enzymes for use in detergents. Genes for a set of derivs. of the ***lipase*** of Humicola lanuginosa with individual amino acids substituted by proline were prepd. by ***PCR*** mutagenesis of the gene and the expression cassette used to manuf. the protein in Aspergillus. An analog of the ***lipase*** with Gly225 replaced by Pro lost 50% of its activity after .apprx.30 min at 70.degree. whereas the wild type lost 50% of its activity after 15 min. at the same temp.

L32 ANSWER 69 OF 81 CAPLUS COPYRIGHT 2002 ACS
1993:78631 Document No. 118:78631 Molecular basis of familial chylomicronemia: Mutations in the lipoprotein ***lipase*** and apolipoprotein C-II genes. Reina, Manuel; Brunzell, John D.; Deeb, Samir S. (Dep. Med., Univ. Washington, Seattle, WA, 98195, USA). J. Lipid Res.,

33(12), 1823-32 (English) 1992. CODEN: JLPRAW. ISSN: 0022-2275.
AB The mol. basis of familial chylomicronemia (type I hyperlipoproteinemia), a rare autosomal recessive trait, was investigated in 6 unrelated individuals (5 of Spanish descent and one of Northern European extn.). DNA amplification by ***PCR*** followed by single strand conformation polymorphism (SSCP) anal. allowed rapid identification of the underlying mutations. Six different mutant alleles (3 of which are previously undescribed) of the gene encoding lipoprotein ***lipase*** (LPL) were discovered in the 5 LPL-deficient patients. These included an 11-bp deletion in exon 2, and 5 missense mutations: Trp 86 Arg (exon 3), His 136 Arg (exon 4), Gly 188 Glu (exon 5), Ile 194 Thr (exon 5), and Ile 205 Ser (exon 5). The Trp 86 Arg mutation is the only known missense mutation in exon 3. The other missense mutations lie in the highly conserved "central homol. ***region*** " in close proximity with the catalytic site of LPL. These and other previously reported missense mutations provide insight into structure/function relationships in the ***lipase*** family. The missense mutations point to the important role of particular highly conserved helixes and beta-strands in proper folding of the LPL mol., and of certain connecting loops in the catalytic process. A nonsense mutation (Arg 19 Term) in the gene encoding apolipoprotein C-II (apoC-II), the cofactor of LPL, was found to underlie chylomicronemia in the 6th patient who had normal LPL but was apoC-II-deficient.

L32 ANSWER 70 OF 81 CAPLUS COPYRIGHT 2002 ACS
1992:568757 Document No. 117:168757 A missense mutation Pro157Arg in lipoprotein ***lipase*** (LPLNijmegen) resulting in loss of catalytic activity. Bruin, Taco; Kastelein, John J. P.; Van Diermen, Denise E.; Ma, Yuanhong; Henderson, Howard E.; Stuyt, Paul M. J.; Stalenhoef, Anton F. H.; Sturk, Augueste; Brunzell, John D.; Hayden, Michael R. (Acad. Med. Cent., Univ. Amsterdam, Amsterdam, 1105, Neth.). Eur. J. Biochem., 208(2), 267-72 (English) 1992. CODEN: EJBCAI. ISSN: 0014-2956.
AB The mol. defect that leads to a deficiency of lipoprotein ***lipase*** (LPL) activity in a proband of Dutch descent is reported. Southern-blot anal. of the LPL gene from the patient did not reveal any major DNA rearrangements. Sequencing of ***polymerase*** - ***chain*** - ***reaction*** -amplified DNA revealed that the proband is a homozygote for G725C, resulting in a substitution of Pro157 for Arg. This substitution alters a restriction site for PvuII, which allowed rapid identification of the mutant allele in family members. Site-directed mutagenesis and transient expression of the mutant LPL in COS cells produced an enzymically inactive protein, establishing the functional significance of this mutation. This naturally occurring mutation which alters the Pro157 adjacent to Asp156 of the proposed catalytic triad, indicates that this ***region*** of the protein is indeed crit. for LPL catalytic activity.

L32 ANSWER 71 OF 81 CAPLUS COPYRIGHT 2002 ACS
1992:525727 Document No. 117:125727 Detection of three separate DNA polymorphisms in the human lipoprotein ***lipase*** gene by gene amplification and restriction endonuclease digestion. Gotoda, Takanari; Yamada, Nobuhiro; Murase, Toshio; Shimano, Hitoshi; Shimada, Masako; Harada, Kenji; Kawamura, Masako; Kozaki, Koichi; Yazaki, Yoshio (Fac. Med., Univ. Tokyo, Tokyo, 113, Japan). J. Lipid Res., 33(7), 1067-72 (English) 1992. CODEN: JLPRAW. ISSN: 0022-2275.
AB A rapid detection method was developed for DNA polymorphisms in the human lipoprotein ***lipase*** (LPL) gene. The examd. polymorphisms include an A-C transversion in the 5'- ***region*** of intron 3, a T-G transversion that occurs within a HindIII site of intron 8, and the previously described C-T transition that causes a PvuII polymorphism in intron 6. Gene fragments encompassing each polymorphic site were amplified by the ***polymerase*** ***chain*** ***reaction*** (***PCR***) and digested with an appropriate restriction enzyme whose recognition site was either naturally affected by the polymorphism or artificially created with a mismatched ***PCR*** -primer. According to the digestion profiles, genotypes were unambiguously distinguished. With this method, resp. allelic frequencies were detd. for 50 or 70 normal subjects. The procedure will facilitate LPL genotyping in the large population.

L32 ANSWER 72 OF 81 CAPLUS COPYRIGHT 2002 ACS
1992:505442 Document No. 117:105442 Cloning and structure of the mono- and

diacylglycerol ***lipase*** -encoding gene from Penicillium camembertii U-150. Yamaguchi, Shotaro; Mase, Tamio; Takeuchi, Kazuyuki (Tsukuba Res. Lab., Amano Pharm. Co., Ltd., Tsukuba, 305, Japan). Gene, 103(1), 61-7 (English) 1991. CODEN: GENED6. ISSN: 0378-1119.

AB A gene (mdlA) encoding mono- and diacylglycerol ***lipase*** (MDGL) from Penicillium camembertii U-150 has been cloned using a 0.9-kb DNA fragment, generated by mixed oligodeoxyribonucleotide (oligo)-primed ***polymerase*** ***chain*** ***reaction*** (***PCR***), as a probe. Comparison of the nucleotide sequence of the gene and its cDNA clone, obtained by ***PCR*** , revealed the presence of two short introns (56 and 53 bp). Two transcription start points (tsp) were localized by primer extension anal. at 37 and 30 bp upstream from the ATG start codon and were preceded by the canonical TATAAA and CAAT sequences. The deduced amino acid (aa) sequence corresponds to 305 aa including a putative signal peptide of 26 aa. Despite significant differences in substrate specificity, the primary structure of the mature ***region*** shows homol. (29% and 40%) to the triacylglycerol ***lipases*** from Mucor miehei and Humicola lanuginosa. Furthermore, the three residues presumed to form the catalytic site, serine, aspartic acid and histidine, are conserved. Primary structure comparisons of MDGL and triacylglycerol ***lipases*** are shown.

L32 ANSWER 73 OF 81 CAPLUS COPYRIGHT 2002 ACS

1992:424223 Document No. 117:24223 A missense (Asp250.fwdarw.Asn) mutation in the lipoprotein ***lipase*** gene in two unrelated families with familial lipoprotein ***lipase*** deficiency. Ishimura-Oka, Kazumi; Semenkovich, Clay F.; Faustinella, Fabrizia; Goldberg, Ira J.; Shachter, Neil; Smith, Louis C.; Coleman, Trey; Hide, Winston A.; Brown, W. Virgil; et al. (Dep. Cell Biol., Baylor Coll. Med., Houston, TX, 77030, USA). J. Lipid Res., 33(5), 745-54 (English) 1992. CODEN: JLPRAW. ISSN: 0022-2275.

AB The authors have identified the mol. basis for familial lipoprotein ***lipase*** (LPL) deficiency in two unrelated families with the syndrome of familial hyperchylomicronemia. All 10 exons of the LPL gene were amplified from the two probands' genomic DNA by ***polymerase*** ***chain*** ***reaction*** . In family 1 of French descent, direct sequencing of the amplification products revealed that the patient was heterozygous for two missense mutations, Gly188 .fwdarw. Glu (in exon 5) and Asp250 .fwdarw. Asn (in exon 6). In family 2 of Italian descent, sequencing of multiple amplification products cloned in plasmids indicated that the patient was a compd. heterozygote harboring two mutations, Arg243 .fwdarw. His and Asp250 .fwdarw. Asn, both in exon 6. Studies using ***polymerase*** ***chain*** ***reaction*** , restriction enzyme digestion (the Gly188 .fwdarw. Glu mutation disrupts an Ava II site, the Arg243 .fwdarw. His mutation, a Hha I site, and the Asp250 .fwdarw. Asn mutation, a Taq I site), and allele-specific oligonucleotide hybridization confirmed that the patients were indeed compd. heterozygous for the resp. mutations. LPL constructs carrying the three mutations were expressed individually in Cos cells. All three mutant LPLs were synthesized and secreted efficiently; one (Asp250 .fwdarw. Asn) had minimal (.apprx.5%) catalytic activity and the other two were totally inactive. The three mutations occurred in highly conserved ***regions*** of the LPL gene. The fact that the newly identified Asp250 .fwdarw. Asn mutation produced an almost totally inactive LPL and the location of this residue with respect to the three-dimensional structure of the highly homologous human pancreatic ***lipase*** suggest that Asp250 may be involved in a charge interaction with an .alpha.-helix in the amino terminal ***region*** of LPL. The occurrence of this mutation in two unrelated families of different ancestries (French and Italian) indicates either two independent mutational events affecting unrelated individuals or a common shared ancestral allele. Screening for the Asp250 .fwdarw. Asn mutation should be included in future genetic epidemiol. studies on LPL deficiency and familial combined hyperlipidemia.

L32 ANSWER 74 OF 81 CAPLUS COPYRIGHT 2002 ACS

1992:16584 Document No. 116:16584 HindIII-polymorphism in the LPL-gene detected by ***PCR*** . Bruin, T.; Reymer, P. W. A.; Groenemeyer, B. E.; Talmud, P. J.; Kastelein, J. J. P. (Div. Hemostasis, Thromb. Atheroscler., Acad. Med. Cent., Amsterdam, 1105 AZ, Neth.). Nucleic Acids Res., 19(22), 6346 (English) 1991. CODEN: NARHAD. ISSN: 0305-1048.

AB Two oligonucleotides derived from the sequences in exon 8 and 9 in the

LPL-gene were designed to amplify the sequence around a polymorphic HindIII-site. This polymorphism is located in intron 8, in contrast to the HindIII-polymorphism in the 3' untranslated ***region*** as described by K. Oka, et al.(1990). The amplified fragment has a size of 1200 bp. The polymorphism in intron 8 was detected upon digestion of the ***PCR*** -product with HindIII. Allele frequencies were Allele H1 (1200 bp)=0.25 and Allele H2 (600 bp)=0.75. The LPL-gene has been located on chromosome 8p22.

L32 ANSWER 75 OF 81 CAPLUS COPYRIGHT 2002 ACS
1991:625431 Document No. 115:225431 Production of heterologous polypeptides by recombinant cattle and transgenic methods. Heyneker, Herbert L.; Deboer, Herman A.; Strijker, Rein; Plantenburg, Gerard; Lee, Sang He (Genpharm International, Inc., USA). PCT Int. Appl. WO 9108216 A1 19910613, 121 pp. DESIGNATED STATES: W: AU, BR, CA, FI, JP, KR, LK, MC, NO, SU; RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US6874 19901130. PRIORITY: US 1989-444745 19891201.
AB A method for prepg. transgenic cows which secrete recombinant proteins into their milk is described. The gene to be expressed in mammary tissue is fused to a mammary tissue-specific promoter, e.g. that of the casein gene, a signal sequence, and a 3' flanking sequence functional in cattle. The chimeric gene is first methylated, e.g. by cloning it in a prokaryotic host. Fertilized oocytes are then transformed with this gene, and the fertilized oocytes are cultured to the preimplantation embryo stage. A cell is removed from the embryo to test for the presence of the desired gene: the chimeric methylated gene is resistant to restriction endonuclease cleavage. The hemiembryo remaining after removing the cell is cloned to prep. multiple embryos which are implanted into a cow to produce transgenic offspring. The milk from the transgenic cows can be used in food formulations, esp. infant formulas. A chimeric gene comprising human lactoferrin cDNA flanked by bovine .alpha.S1-casein promoter and signal sequence and 3' ***regions*** was prepd. Transgenic cows secreting lactoferrin into their milk were produced using this gene according to the above procedure.

L32 ANSWER 76 OF 81 CAPLUS COPYRIGHT 2002 ACS
1991:443165 Document No. 115:43165 Assignment of the human pancreatic colipase gene to chromosome 6p21.1 to pter. Davis, Richard C.; Xia, Yurong; Mohandas, T.; Schotz, Michael C.; Lusis, Aldons J. (VA Wadsworth Med. Cent., Los Angeles, CA, 90073, USA). Genomics, 10(1), 262-5 (English) 1991. CODEN: GNMCEP. ISSN: 0888-7543.
AB Pancreatic colipase is a 12-kDa polypeptide cofactor for pancreatic ***lipase*** (EC 3.1.1.3), an enzyme essential for the absorption of dietary long-chain triglyceride fatty acids. Colipase is thought to anchor ***lipase*** noncovalently to the surface of lipid micelles, counteracting the destabilizing influence of intestinal bile salts. Primers derived from the known amino acid sequence, were used in the ***polymerase*** ***chain*** ***reaction*** to produce a cDNA clone corresponding to the complete coding ***region*** of the human procolipase mRNA. Southern blot anal. of genomic DNA from a panel of mouse-human somatic cell hybrids indicated that the colipase gene (CLPS) resides on human chromosome 6. Further anal. of somatic cell hybrids carrying chromosome 6 translocations permitted ***regional*** localization of CLPS to the 6p21.1-pter ***region*** .

L32 ANSWER 77 OF 81 CAPLUS COPYRIGHT 2002 ACS
1991:402212 Document No. 115:2212 Molecular basis of lipoprotein ***lipase*** deficiency in two Austrian families with type I hyperlipoproteinemia. Paulweber, Bernhard; Wiebusch, Heiko; Miesenboeck, Gero; Funke, Harald; Assmann, Gerd; Hoelzl, Bertram; Sippl, Manfred J.; Friedl, Walter; Patsch, Josef R.; Sandhofer, Friedrich (1st Dep. Med., Landeskrankenanstalten Salzburg, Salzburg, A-5020, Austria). Atherosclerosis (Shannon, Irel.), 86(2-3), 239-50 (English) 1991. CODEN: ATHSBL. ISSN: 0021-9150.
AB To det. the mol. basis for type I hyperlipoproteinemia in 2 Austrian families, the lipoprotein ***lipase*** (LPL) gene of 2 patients exhibiting LPL deficiency was analyzed by Southern blotting and by direct genomic sequencing of DNA amplified by ***polymerase*** ***chain*** ***reaction*** (***PCR***). All exons of the LPL gene except part of the noncoding ***region*** of exon 10, all splice donor and

acceptor sites, as well as 430 basepairs of the 5'- ***region*** including the promotor were sequenced. A homozygous substitution of adenine for guanine in the fifth exon at cDNA position 818 of the LPL gene was found. The base change described abolishes a normally present AvaII restriction site allowing the identification of carriers of the mutant allele by AvaII digestion of ***PCR*** fragments of exon 5; three members of the 2 families were homozygous for this mutation and 10 members were heterozygous. The activity of LPL in postheparin plasma was almost completely absent in homozygotes and about half normal in heterozygotes. The loss of activity was related to LPL protein structure. This mutation alters the amino acid sequence at residue 188 from Gly to Gly. The conformational preferences of the protein chain around position 188 were calcd. with the use of a knowledge-based computerized method. The most probable conformation is a beta-turn formed by residues 189-192. The mutation destabilizes the beta-turn and/or a larger domain crit. for substrate alignment.

L32 ANSWER 78 OF 81 CAPLUS COPYRIGHT 2002 ACS

1991:200905 Document No. 114:200905 Familial chylomicronemia (type I hyperlipoproteinemia) due to a single missense mutation in the lipoprotein ***lipase*** gene. Ameis, Detlev; Kobayashi, Junji; Davis, Richard C.; Ben-Zeev, Osnat; Malloy, Mary J.; Kane, John P.; Lee, Gregory; Wong, Howard; Havel, Richard J.; Schotz, Michael C. (Veterans Adm. Wadsworth Med. Cent., Los Angeles, CA, 90073, USA). J. Clin. Invest., 87(4), 1165-70 (English) 1991. CODEN: JCINAO. ISSN: 0021-9738.

AB Complete deficiency of lipoprotein ***lipase*** (LPL) causes the chylomicronemia syndrome. To understand the mol. basis of LPL deficiency, 2 siblings with drastically reduced postheparin plasma lipolytic activities were selected for anal. of their LPL gene. The ***polymerase*** ***chain*** ***reaction*** was used to examine the 9 coding LPL exons in the 2 affected siblings and 3 relatives. DNA sequence anal. revealed a single nucleotide change compared with the normal LPL cDNA: a G .fwdarw. A substitution at nucleotide position 680. This transition caused a replacement of glutamic acid for glycine at amino acid residue 142 of the mature LPL protein. Amino acid sequence comparisons of the ***region*** surrounding glycine-142 indicated that it is highly conserved among ***lipases*** from different species, suggesting a crucial role of this domain for the LPL structure. Expression studies of the mutant LPL cDNA in COS-7 cells produced normal amts. of enzyme mass.. However, the mutated LPL was not catalytically active, nor was it efficiently secreted from the cells. This established that the Gly .fwdarw. Glu substitution at amino acid 142 is sufficient to abolish enzymic activity and to result in the chylomicronemia syndrome obsd. in these patients.

L32 ANSWER 79 OF 81 CAPLUS COPYRIGHT 2002 ACS

1991:76248 Document No. 114:76248 Identification of two separate allelic mutations in the lipoprotein ***lipase*** gene of a patient with the familial hyperchylomicronemia syndrome. Dichek, Helen L.; Fojo, Silvia S.; Beg, Obaid U.; Skarlatos, Sonia I.; Brunzell, John D.; Cutler, Gordon B., Jr.; Brewer, H. Bryan, Jr. (Mol. Dis. Branch, Natl. Heart, Lung, Blood Inst., Bethesda, MD, 20892, USA). J. Biol. Chem., 266(1), 473-7 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB The mol. defects resulting in a deficiency of lipoprotein ***lipase*** (I) activity in a patient with the familial hyperchylomicronemia syndrome were identified. Increased I mass but undetectable I activity in the patient's post-heparin plasma indicate the presence of an inactive enzyme. No major gene rearrangements were identified by Southern blot anal. of the patient's I gene and Northern blot hybridization revealed a I mRNA of normal size. Sequence anal. of ***polymerase*** ***chain*** ***reaction*** -amplified I cDNA identified 2 sep. allelic mutations. A T-to-C transition at nucleotide 836 results in the substitution of Ile194, located near the putative interfacial recognition site of I, to a Thr. A G-to-A mutation at base 983 leads to the substitution of a His for Arg243 and the loss of a HhaI restriction enzyme site. Arg243 is near His241, which has been postulated to be part of the catalytic triad of I. Direct sequencing of amplified cDNA and digestion with HhaI established that the proband is a compd. heterozygote for each base substitution. Transient expression of each of the mutant I cDNAs in human embryonal kidney-293 cells resulted in the synthesis of enzymically inactive proteins, establishing the functional significance of the mutations. Thus, the

Ile194 to Thr194 and Arg243 to His243 substitutions occur in I ***regions*** - essential for normal enzyme activity and each mutation results in the expression of a nonfunctional enzyme leading to the hyperchylomicronemia syndrome manifested in the proband.

L32 ANSWER 80 OF 81 CAPLUS COPYRIGHT 2002 ACS
1991:76176 Document No. 114:76176 ***PCR*** assay for a polymorphic PvuII site in the LPL gene. Johnson, J. P.; Nishina, P. M.; Naggert, J. K. (Med. Cent., Children's Hosp., Oakland, CA, 94609, USA). Nucleic Acids Res., 18(24), 7469 (English) 1990. CODEN: NARHAD. ISSN: 0305-1048.
AB The ***polymerase*** ***chain*** ***reaction*** was used to detect a polymorphic PvuII site in the human lipoprotein ***lipase*** gene on chromosome 8. Two oligonucleotides derived from the sequence flanking the polymorphic PvuII site in the intron between exon 6 and 7 of the gene were used to amplify the ***region*** and generate a fragment of the predicted size of 319 base pairs (bp). The frequency of occurrence of the PuuII site was 0.43 among 15 samples. ***PCR*** conditions and modifications are presented.

L32 ANSWER 81 OF 81 CAPLUS COPYRIGHT 2002 ACS
1990:402534 Document No. 113:2534 Lipoprotein lipaseBethesda: a single amino acid substitution (Ala176 .fwdarw. Thr) leads to abnormal heparin binding and loss of enzymic activity. Beg, Obaid U.; Meng, Martha S.; Skarlatos, Sonia I.; Previato, Lorenzo; Brunzell, John D.; Brewer, H. Bryan, Jr.; Fojo, Silvia S. (Mol. Dis. Branch, Natl. Heart, Lung Blood Inst., Bethesda, MD, 20892, USA). Proc. Natl. Acad. Sci. U. S. A., 87(9), 3474-8 (English) 1990. CODEN: PNASA6. ISSN: 0027-8424.
AB The mol. defect that leads to a deficiency of lipoprotein ***lipase*** (LPL) activity in the proband from a Bethesda kindred was identified. The pre- and post-heparin plasma LPL mass in the proband was elevated when compared to controls; however, there was no detectable LPL activity, indicating the presence of a defective enzyme (termed LPLBethesda). Anal. of the patient's post-heparin plasma by heparin-Sepharose affinity chromatog. demonstrated that the mutant LPL had an altered affinity for heparin. Southern blot hybridization of the gene for LPLBethesda revealed no major rearrangements. Northern blot anal. of LPLBethesda mRNA from patient monocyte-derived macrophages revealed normal-sized mRNAs (3.4 and 3.7 kilobases) as well as normal cellular mRNA levels when compared to control macrophages. Sequence anal. of ***polymerase*** ***chain*** ***reaction*** (***PCR***)-amplified PLP cDNA revealed a guanine .fwdarw. adenine substitution at position 781 of the normal LPL gene that resulted in the substitution of an alanine (Ala) for a threonine (Thr) at residue 176 and the loss of a restriction endonuclease SfaNI site present in the normal LPL gene. Amplification of cDNA by the ***PCR*** followed by digestion with SfaNI established that the patient was a true homozygote for the mutation. Expression of LPL cDNA in COS-7 cells resulted in the synthesis of a nonfunctional LPL enzyme establishing that the Ala .fwdarw. Thr substitution was the mutation responsible for the inactive LPL. The indentification of this mutation in the LPL gene defined a ***region*** of the LPL enzyme, at Ala-176, that was essential for normal heparin-binding and catalytic activity. It was proposed that an amino acid substitution in this crit. ***region*** of LPLBethesda results in the synthesis of a nonfunctional enzyme that leads to the chylomicronemia syndrome expressed in this proband.

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=> E REETZ M/AU
=> S E3-E8
             6 "REETZ M"/AU
           100 "REETZ M T"/AU
             1 "REETZ MANDRED T"/AU
            10 "REETZ MANFRED"/AU
           259 "REETZ MANFRED T"/AU
             8 "REETZ MANFRED THEODOR"/AU
L33        384 ("REETZ M"/AU OR "REETZ M T"/AU OR "REETZ MANDRED T"/AU OR "REET
               Z MANFRED"/AU OR "REETZ MANFRED T"/AU OR "REETZ MANFRED THEODOR"
               /AU)

=> E ZONTA A/AU
=> S E3,E4
            21 "ZONTA A"/AU
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            10 "ZONTA ALBIN"/AU
L34         31 ("ZONTA A"/AU OR "ZONTA ALBIN"/AU)

=> E SCHIMOSSEK K/AU
=> S E3,E4
             1 "SCHIMOSSEK K"/AU
             7 "SCHIMOSSEK KLAUS"/AU
L35          8 ("SCHIMOSSEK K"/AU OR "SCHIMOSSEK KLAUS"/AU)

=> E LIEBETON K/AU
=> S E3,E4
             1 "LIEBETON K"/AU
             9 "LIEBETON KLAUS"/AU
L36         10 ("LIEBETON K"/AU OR "LIEBETON KLAUS"/AU)

=> S E3,E11
          7270 "E3"/BI
          1214 "E11"/BI
             1 "E3,E11"/BI
                 (("E3"(W)"E11")/BI)
             2 E300TS/BI
L37          3 ("E3,E11"/BI OR E300TS/BI)

=> E JAGER K/AU
=> S E3,E11
             8 "JAGER K"/AU
             1 "JAGER KARL"/AU
L38          9 ("JAGER K"/AU OR "JAGER KARL"/AU)

=> S L33,L34,L35,L36,L38
L39        418 (L33 OR L34 OR L35 OR L36 OR L38)

=> S L39 AND L15
L40          8 L39 AND L15

=> S L40 NOT (L22,L27,L32)
L41          4 L40 NOT ((L22 OR L27 OR L32))

=> D 1-4 CBIB ABS

L41  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2002 ACS
2000:119638   Document No. 132:275952  Enantioselective enzymes for organic
     synthesis created by directed evolution.    ***Reetz, Manfred T.***  ;
     Jaeger, Karl-Erich (Max-Planck-Institut fur Kohlenforschung, Mulheim an
     der Ruhr, 45470, Germany).  Chemistry--A European Journal, 6(3), 407-412
     (English) 2000.  CODEN: CEUJED.  ISSN: 0947-6539.  Publisher: Wiley-VCH
     Verlag GmbH.
AB   A new concept for the creation of enzymes displaying improved
     enantioselectivity in a given reaction is described; it is based on
     "evolution in the test tube".  Accordingly, proper mol. biol. methods for
     random mutagenesis, gene expression, and high-throughput screening systems
     for the rapid assay of enantioselectivity are combined.  Several rounds of
     mutagenesis and screening are generally necessary in order to create
     mutant enzymes that show high degrees of enantioselectivity, as in the
     case of the lipase-catalyzed hydrolytic kinetic resoln. of a chiral ester
     in which the original enantioselectivity of 2% ee (E = 1) increases to >
     90% ee (E =25).

L41  ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2002 ACS
1999:81691   Document No. 130:149528  Mutagenic   ***PCR***   in preparation
     and identification of new hydrolases with improved characteristics.
       ***Reetz, Manfred T.***  ;   ***Zonta, Albin***  ;   ***Schimossek,***
  ***     Klaus***  ;   ***Liebeton, Klaus***  ; Jaeger, Karl-Erich
     (Studiengesellschaft Kohle m.b.H., Germany).  Ger. Offen. DE 19731990 A1
     19990128, 48 pp.  (German).  CODEN: GWXXBX.  APPLICATION: DE 1997-19731990
     19970725.
AB   Hydrolase genes are mutated by mutagenic   ***PCR***  , the mutated genes
     are fragmented enzymically then enzymically reassembled, the (scrambled)
     mutated genes are expressed in microorganisms, and the characteristics of
     the mutant enzymes are detd.  The mutagenicity of the   ***PCR***
     process is adjusted by varying the Mg2+, Mn2+, and dNTP concns.  The
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lipase lipA gene of Pseudomonas aeruginosa was subjected to the above process. A screening test using (R,S)-2-methyldecanoic acid p-nitrophenyl ester identified mutants with improved enantioselectivity (S-isomer preferred).

L41 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2002 ACS
1999:53339 Document No. 130:233836 Superior biocatalysts by directed evolution. ***Reetz, Manfred T.*** ; Jaeger, Karl-Erich (Max-Planck-Institut fur Kohlenforschung, Mulheim/Ruhr, D-45470, Germany). Topics in Current Chemistry, 200(Biocatalysis: From Discovery to Application), 31-57 (English) 1999. CODEN: TPCCAQ. ISSN: 0340-1022. Publisher: Springer-Verlag.
AB A review with 83 refs. Useful biocatalysts for org. chem. can be created by directed evolution. Mutations are introduced into genes encoding biocatalyst proteins of interest by error-prone ***PCR*** or other random mutagenesis methods. The mutated genes can be rearranged by recombinative processes like DNA shuffling, thereby significantly enhancing the efficiency with which genes can be evolved. These genes are expressed in suitable microbial hosts leading to the prodn. of functional biocatalysts. Selection or screening procedures serve to identify in a large library of potential candidates the biocatalyst which possesses the desired properties. Examples of applications include subtilisin E with greatly improved catalytic activity and stability in org. solvent, an esterase with 50-fold higher activity in org. solvent, and a .beta.-lactamase conferring a 32,000-fold increased antibiotic resistance. Furthermore, directed evolution of a bacterial lipase resulted in a significant increase in enantioselectivity, thereby demonstrating the enormous potential of this process for org. chem.

L41 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS
1998:466880 Document No. 129:148083 Overexpression, immobilization and biotechnological application of Pseudomonas lipases. ***Reetz, Manfred*** *** T.*** ; Jaeger, Karl-Erich (Max-Planck-Institut fur Kohlenforschung, Mulheim, D-45470, Germany). Chemistry and Physics of Lipids, 93(1-2), 3-14 (English) 1998. CODEN: CPLIA4. ISSN: 0009-3084. Publisher: Elsevier Science Ireland Ltd..
AB A review with 75 refs. Pseudomonas lipases play an important role in biotechnol. both as hydrolases for detergent additives and as synthases catalyzing the kinetic resoln. of racemic compds. Large-scale prodn. of Pseudomonas lipases requires correct folding and secretion through the bacterial membranes. Controllable expression of the gene lipH encoding a lipase-specific foldase proves to be important for overexpression in the homologous host Escherichia coli. Construction of appropriate His-tagged fusion proteins permitted overexpression, secretion and one-step purifn. of lipase from culture supernatants of the homologous host Pseudomonas aeruginosa. The immobilization of lipases in hydrophobic sol-gel materials derived from alkylsilane precursors of the type RSi(OCH3)3 or mixts. of RSi(OCH3)3 and Si(OCH3)4 provides highly active chem. and thermally stable heterogeneous biocatalysts. The entrapped lipases are excellent catalysts in a variety of synthetic org. transformations. Using directed evolution based on error prone ***PCR*** , the enantioselectivity of the hydrolysis of a chiral ester, catalyzed by the lipase from P. aeruginosa, can be increased from ee 2 to ee 81% in just four mutagenesis cycles.